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Evaluation of biogenic amines and microbial counts throughout the ripening of goat cheeses from pasteurized and raw milk

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The effect of the hygienic quality of milk on changes in microbial counts and biogenic amine content was evaluated during ripening of goat cheeses manufactured from pasteurized and raw milks at 1, 14, 30, 60 and 90 d. The original milk, rennet, curd and whey were also included in the study. The pH, salt content and extent of proteolysis in the cheese were also evaluated. Spermidine and spermine were the main amines in raw milk, while they were minor amines in cheeses. Other amines increased markedly during ripening, tyramine being the main amine in cheese made from raw milk and cadaverine and putrescine in those produced from pasteurized milk. *Enterobacteriaceae* counts decreased during ripening whereas those of lactic acid bacteria increased, especially lactobacilli and enterococci. Cheese made from raw milk showed higher microbial counts during ripening than those made from pasteurized milk, especially for *Enterobacteriaceae* and enterococci, counts being 2 or 3 log units higher. Raw milk cheese showed remarkably higher biogenic amines compared with pasteurized milk cheeses. Therefore, pasteurization of milk causes a decrease in final biogenic amine content of cheese as a result of the reduction of its microbial counts.

Keywords: Biogenic amines, goat cheese, pasteurized milk, raw milk.

Some biogenic amines in cheese may arise from decarboxylation of amino acids by microorganisms (Joosten & Olieman, 1986), but others can be natural (Bardócz, 1995). The presence of biogenic amines in cheeses has been investigated previously (Vale & Glória, 1998; Durlo-Özkaya et al. 2000; Novella-Rodríguez et al. 2000; Valsamaki et al. 2000).

The main biogenic amines in cheese coming from microbial activity are: (a) aromatic amines, such as tyramine (TY) and histamine (HI); (b) the diamines, cadaverine (CA) and putrescine (PU); and (c) other aromatic amines, usually found at lower concentrations or scarcely present in cheese, such as β-phenylethylamine (PHE) and tryptamine (TR), octopamine (OC), dopamine (DO) and serotonin (SE). Due to their bacterial origin, these amines could be used as an indicator of the hygienic quality of food (Vidal-Carou et al. 1990; Veciana-Nogués et al. 1996; Schneller et al. 1997). However, cheese also contains agmatine (AG), spermidine (SD) and spermine (SM), which are polyamines not produced by bacterial decarboxylation (Bardócz, 1995).

Biogenic amines in cheese could be a result of the decarboxylase activity of the fermentative microflora. However, these amines may also arise from the microbial activity of raw milk microbial contaminants during cheese making (Hernández-Jover et al. 1996). A high concentration of these amines could be used as an indicator of the hygienic quality of cheese (Scheneller et al. 1997).

Pasteurization is the heat-treatment most often used for cheese making to avoid pathogenic microorganisms and to reduce other contaminant microorganisms present in milk and it is also an effective tool to reduce biogenic amine contents in cheeses. In fact, biogenic amine content of cheeses from pasteurized milk is in general much lower than those of raw milk (Stratton et al. 1991; Schneller et al. 1997; Novella-Rodriguez et al. 2003).

The interest in biogenic amines, in particular TY and HI, lies in their potential detrimental effects on consumers (Silla-Santos, 1996). Under normal conditions exogenous amines from the diet are detoxified by conjugation.

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However, dietary biogenic amines may accumulate in the body when detoxification processes are genetically deficient (sensitive individuals), or when amine oxidase inhibitors, such as several drugs, or alcohol are present or where various gastrointestinal diseases occur (Mariné-Font et al. 1995; Lehane & Olley, 2000). Undesirable physiological effects such as headaches, hot flushes, respiratory distress, hypo- or hypertension, cardiac palpitation and possibly shock could be related to aromatic amines (Murray et al. 1982). CA and PU may produce carcinogenic nitrosamines after reaction with nitrites (Scanlan, 1983). In contrast, polyamines fulfil a physiological role, since they are necessary for cell proliferation.

Several studies have addressed the effects of the treatment of milk on the accumulation of biogenic amines in cheese made from cow and ewe milk (Joosten, 1988; Ordoñez et al. 1997; Schneller et al. 1997). In general, there is a greater consumption of cheese made from cow milk; however, in the Mediterranean, homemade style cheese made from goat milk is common. Nevertheless, there are few data on the occurrence of biogenic amines in goat cheese, or the factors affecting their formation, with the exception of data reported by Tham et al. (1990) and Novella-Rodríguez et al. (1999).

The investigation was aimed to study the effect of milk treatment on the biogenic amine profile of goat cheeses during ripening. We compare the biogenic amine profiles in hard non-cooked pressed cheese made from raw and from pasteurized goat milk. Biogenic amines, microbial counts and proteolytic parameters were monitored throughout the cheese-making process. Special emphasis was placed to check whether there are differences in one or more specific amines between both kinds of cheeses. In addition, the microflora present in the cheese-making plant, such as equipment, tools, materials and environment, was also studied to better understand the origin genesis of biogenic amine.

Materials and Methods

Cheese manufacture

Two batches of goat cheese were manufactured in parallel using pasteurized and raw milk in three separate trials. The milk was pasteurized at 72 °C for 15 s, and raw milk was refrigerated at 4 °C for a maximum of 24 h before cheese manufacture. Cheese making was performed in the Pilot Plant of the Food Technology Unit – Centre de Referència en Tecnologia dels Aliments (CeRTA) at the Autonomous University of Barcelona. A lactic starter (*Lactococcus lactis* subsp. *lactis* plus *Lactococcus lactis* subsp. *cremoris*; MAO 16, Texel, Larbus S.A., Madrid, Spain), previously grown in sterile milk (reconstituted skimmed milk powder, 120 g/l), was inoculated (20 ml/kg) in milk (100 kg) at 32 °C. This starter was unable to produce biogenic amines *in vitro* (Novella-Rodríguez et al. 2002). Prior to the addition of the starter, raw milk (in the churns used for transport) was placed in a water bath at 50 °C until the milk reached 32 °C. Next, calf rennet (0·2 ml/kg, containing 780 mg chymosin/l (Renifor-15/E, Lamirsa, Barcelona, Spain), and 3·5 g calcium chloride/kg (0·0025 ml/kg)) were added as coagulating agents. Following coagulation (40 min), the curd was cut in very small pieces (<1 cm^3), held at 32 °C for 15 min, and stirred to facilitate subsequent draining. Drained curds were moulded and pressed in a pneumatic press (cylindrical moulds of 13·6 × 13·2 cm) at 1·3 kPa for 1 h and at 2·6 kPa for 13 h at 14 °C. Salting was performed by immersion in brine (190 g NaCl/l solution) for 6 h. Unwrapping cheeses (12 cheeses of approximately 1·3 kg from each batch) were ripened at 14 °C and at a relative humidity of around 85%.

Sampling

Samples were taken from raw materials (milk, calcium chloride, rennet and brine), curd, whey and cheese after 1, 14, 30, 60 and 90 d ripening. Cheeses were sampled according to International Dairy Federation standard (1995). A whole cheese was used for each sampling point. Cheeses were divided in three radial portions, one for microbial analyses, another for determination of biogenic amines and the last for other physicochemical analyses. Furthermore, several potential contamination points for the production of biogenic amines were studied, taking samples from vat, churns, wires, pressure plates, strainers, moulds, cheese clothes, and gloves in order to determine their microbial counts. Likewise, five plates of Plate Count Agar were used to control the environmental air conditions during cheese manufacture. One was placed in a heat exchanger, three in the coagulation chamber, and one in ripening chamber. Analyses were always performed in duplicate.

Biogenic amines determination

The amine contents of the samples were measured by HPLC, as described by Novella-Rodríguez et al. (2000). Twelve amines were quantified: tyramine (TY), histamine (HI), β-phenylethylamine (PHE), tryptamine (TR), cadaverine (CA), putrescine (PU), agmatine (AG), spermidine (SD), spermine (SM), octopamine (OC), dopamine (DO), and serotonin (SE). All results were expressed in mg/kg dry matter.

Microbiological analysis

Samples (10 g or 10 ml) were placed in sterile Stomacher bags and homogenized in a Stomacher Lab-blender 400 (Seward Medical, London, UK) for 2 min in 90 ml of buffered peptone water (Oxoid Ltd, Basingstoke, Hampshire, UK) with 10 g Tween 80/l (Liofilchem, Roseto degli Abruzzi, TE, Italy). Serial decimal dilutions were prepared from 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

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Table 1. Microbial counts [log (cfu/cm^2)] of selected equipment, tools and materials used in cheese making

Values are means ± SD for $n=6$

Sample	Vat	Churn	Wires	Pressure Plates	Strainers	Moulds	Cheese Clothes	Gloves
PCA†	0·98 ± 0·01	4·07 ± 0·02	1·66 ± 0·01	1·09 ± 0·01	0·55 ± 0·03	2·84 ± 0·03	1·48 ± 0·02	nd
LAC	nd	4·92 ± 0·01	2·09 ± 0·02	0·65 ± 0·01	nd	3·33 ± 0·03	1·74 ± 0·02	nd
LAB	nd	nd	nd	nd	nd	1·72 ± 0·02	nd	nd
ENT	nd	4·24 ± 0·01	nd	nd	nd	nd	nd	nd
ENC	nd	nd	nd	nd	nd	nd	nd	nd

†PCA, aerobic mesophilic microorganisms; LAC, lactococci; LAB, lactobacilli; ENT, *Enterobacteriaceae*; ENC, enterococci
nd, not detected: for ENT, <100 (cfu/cm^2); for PCA, LAC, LAB and ENC <10 (cfu/cm^2)

plates (Oxoid) supplemented with lactose (Scharlau Microbiology, Barcelona, Spain) and incubated for 48 h at 30 °C, lactobacilli on Rogosa agar (Oxoid) incubated at 30 °C for 5 d in an atmosphere containing 10% CO_2, enterococci on Kanamicin Aesculin Azide agar (KAA, Oxoid) at 37 °C for 48 h, *Enterobacteriaceae* on Violet Red Bile Glucose agar (VRBG, Biokar Diagnostics, Beuvais, France) incubated with a double layer for 24 h at 37 °C.

The microbial analysis of equipment, tools and materials was performed by swabbing an area from 10 to 100 cm^2, depending on the kind of surface, with a sterile cotton swab rinsed in 0·1% peptone water. Swabs were incubated before preparing the decimal dilutions in 0·1% peptone water for 15 min at 37 °C, then they were mechanically shaken for 1 min with a Vortex.

Physicochemical analysis

Samples of cheeses were analysed for dry matter and total nitrogen (TN) following standard methods (International Dairy Federation, 1982, 1993). The pH of a cheese/distilled water (1 : 1) slurry was measured. Salt (S) was determined by chloride analysis (Corning 926 Salt Analyser; Corning Medical and Scientific Glass Works, Medfield, MA). Water-soluble fractions of the cheeses were prepared according to the method of Kuchroo & Fox (1982). The pH 4·6-soluble nitrogen (WSN) were prepared from water-soluble fractions and determined by the Kjeldahl method (International Dairy Federation, 1993). WSN/TN was used as proteolysis index. All the analyses were performed by duplicate.

Statistical analysis

The values of microbial counts and biogenic amine contents did not show a normal distribution; therefore the Wilcoxon test for non-parametric data was applied. To determine the relationship between times of ripening, microbial counts, biogenic amines and proteolytic parameters, linear regression analysis was used. Statistical analysis was performed using the SPSS package for Windows 9.0 (SPSS Inc, Chicago IL, USA). All results are expressed as the average values (standard deviation) from samples corresponding to the three productions.

Results and Discussion

The analysis of potential contamination points showed, in general, the microbial counts of the equipment was low, but the churns were a great source of microbial contamination, especially for *Enterobacteriaceae* (Table 1). In the pilot plant, as in factories, an extensive cleaning programme with acid and alkaline detergents is applied, but churns are easily contaminated during the return to the farm and/or during milk churn collection. Regarding samples of environment, the highest counts were obtained in the ripening chamber and the lowest in the coagulation chamber (data not shown).

Among the raw materials used in cheese making, microorganisms and biogenic amines were absent only in calcium chloride. In rennet, few aerobic mesophilic microorganisms [0·70 ± 0·01 log (cfu/ml)] were found, average levels of TY being near to 30 mg/kg and near to 8–10 mg/kg for HI, CA and PU. However, because the dilution effect involved in cheese making (20 ml rennet per 100 kg milk were added) the direct contribution of rennet to the final amine content in the cheese should be negligible. Brine showed counts of 4·74 ± 0·10 log (cfu/ml) for aerobic mesophilic microorganisms, 5·02 ± 0·10 log (cfu/ml) for *lactococci*, and 0·43 ± 0·01 log (cfu/ml) for *Enterobacteriaceae*, were found. Some of the microorganisms belonging to these groups have been reported as amine-forming bacteria. However in brine only low levels of CA and PU were found. The milk additives were not important contributors to the microbial counts and biogenic amines in the initial mixtures and thus their influence on the final amine contents was minimal.

Lactobacilli, *Enterobacteriaceae* and enterococci were not found in pasteurized milk, which showed remarkably lower microbial counts ($P<0·05$) than raw milks (Table 2). In addition, in raw milk the microbial growth was favoured by the warning to 32 °C, prior to starter addition. Although microbial counts were clearly higher in raw milk, the biogenic amines levels were relatively similar in both pasteurized and raw milks. The only amines detected were polyamines (SD and SM) and CA. Polyamines are physiological amines that do not result from bacterial metabolism (Bardócz, 1995), while CA is mainly related to growth of microorganisms (Stratton et al. 1991; Petridis & Steinhart,

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Table 2. Biogenic amines (mg/kg dry matter) and microbial counts of milk [log (cfu/ml)] and curd [log (cfu/g)]

Values are means ± SD for $n = 6$

	Milks			Curds from pasteurized or raw milk	
	Pasteurized	Raw at 4 °C	Raw at 32 °C	Pasteurized milk	Raw milk
PCA†	$2{\cdot}92 \pm 0{\cdot}23^{A}$	$4{\cdot}46 \pm 0{\cdot}39^{B}$	$4{\cdot}94 \pm 0{\cdot}01^{B}$	$8{\cdot}15 \pm 0{\cdot}80^{C}$	$7{\cdot}53 \pm 0{\cdot}76^{C}$
LAC	$3{\cdot}33 \pm 0{\cdot}33^{A}$	$4{\cdot}90 \pm 0{\cdot}46^{A}$	$6{\cdot}95 \pm 0{\cdot}01^{B}$	$7{\cdot}64 \pm 0{\cdot}79^{B}$	$7{\cdot}97 \pm 0{\cdot}79^{B}$
LAB	nd^{A}	$3{\cdot}65 \pm 0{\cdot}36^{B}$	$2{\cdot}26 \pm 0{\cdot}01^{C}$	nd^{A}	$3{\cdot}23 \pm 0{\cdot}32^{B}$
ENT	nd^{A}	$2{\cdot}11 \pm 0{\cdot}21^{B}$	$3{\cdot}60 \pm 0{\cdot}01^{C}$	$3{\cdot}24 \pm 0{\cdot}32^{C}$	$4{\cdot}74 \pm 0{\cdot}48^{D}$
ENC	nd^{A}	$2{\cdot}24 \pm 0{\cdot}25^{B}$	$2{\cdot}93 \pm 0{\cdot}01^{B}$	$1{\cdot}52 \pm 0{\cdot}18^{C}$	$3{\cdot}81 \pm 0{\cdot}38^{D}$
CA	nd^{A}	$0{\cdot}13 \pm 0{\cdot}03^{A}$	$0{\cdot}12 \pm 0{\cdot}01^{A}$	$0{\cdot}49 \pm 0{\cdot}01^{B}$	$2{\cdot}40 \pm 0{\cdot}08^{C}$
PU	nd^{A}	nd^{A}	nd^{A}	nd^{A}	$3{\cdot}38 \pm 0{\cdot}05^{B}$
SD	nd^{A}	$0{\cdot}10 \pm 0{\cdot}01^{A}$	$0{\cdot}11 \pm 0{\cdot}02^{A}$	$1{\cdot}65 \pm 0{\cdot}01^{B}$	$1{\cdot}82 \pm 0{\cdot}40^{B}$
SM	$0{\cdot}83 \pm 0{\cdot}01^{A}$	$0{\cdot}86 \pm 0{\cdot}08^{A}$	$0{\cdot}86 \pm 0{\cdot}09^{A}$	$0{\cdot}86 \pm 0{\cdot}08^{A}$	$0{\cdot}89 \pm 0{\cdot}05^{A}$

†PCA, aerobic mesophilic microorganisms; LAC, lactococci; LAB, lactobacilli; ENT, *Enterobacteriaceae*; ENC, enterococci; TY, tyramine; HI, histamine; CA, cadaverine; PU, putrescine; SD, spermidine; SM, spermine

nd, not detected: for content of biogenic amine nd means <0·05 mg/kg; for ENT, <100 cfu/ml or <100 cfu/g; for PCA, LAC, LAB and ENC <10 cfu/ml or log cfu/g

[A]Values in each row with common superscripts were not significantly different ($P > 0{\cdot}05$)

1996; Sharaf et al. 1997). Although warming at 32 °C favoured the growth of microorganisms, no associated increase was detected in biogenic amine content, which is consistent with previous studies (ten Brink et al. 1990; Bardócz, 1993; Petridis & Steinhart, 1996; Schneller et al. 1997; Novella-Rodríguez et al. 2002).

The counts of aerobic mesophilic microorganisms and lactococci, in curds from pasteurized and from raw milk, were similar because the inoculum (2%) of starter was the same for both. However, lower microbial counts of lactobacilli, *Enterobacteriaceae* and enterococci were found in curds from pasteurized than from raw milk. Lactobacilli were found only in curd from raw milk indicating that these bacteria came from the raw milk. On the contrary the occurrence of *Enterobacteriaceae* and enterococci in curd from pasteurized milk (though not in milk) indicates a contamination during cheese making after the pasteurization (Joosten & Northolt, 1987; Petridis & Steinhart, 1996; Leuschner et al. 1999). According to their physiological origin, occurrence of SD and SM in curd can only be explained by their presence in milk and, as expected, no differences in SD and SM contents between curds from pasteurized and raw milk were found. The higher content of these two polyamines in curd than in milk was due to the concentration effect that occurs during coagulation. Most of the biogenic amines present in milk remained in the curd. Thus, among biogenic amines only CA was detected in whey from raw milk (data not shown), the amounts being 5-fold lower than the contents found in curd.

CA and PU were statistically higher in curds from raw milk than in those from pasteurized milk in agreement with its higher microbial counts. A great formation of biogenic amines could be attributable to poor hygienic quality of the milk, which is mainly related to the growth of *Enterobacteriaceae* and enterococci (Joosten & Northolt, 1987; Petridis & Steinhart, 1996; Leuschner et al. 1999).

The microbial counts (Table 3) throughout ripening were also lower in cheeses from pasteurized milk than in those from raw milk, the difference being statistically significant ($P < 0{\cdot}05$) for lactobacilli, *Enterobacteriaceae* and enterococci. Aerobic mesophilic microorganisms and lactococci showed a similar profile throughout ripening of both cheeses from pasteurized and from raw milk. At the beginning of ripening, lactobacilli, *Enterobacteriaceae* and enterococci counts in cheese made from raw milk were 2 or 3 log units higher than those from pasteurized milk in agreement with the higher microbial counts in raw milk. *Enterobacteriaceae* counts decreased during ripening in both kinds of cheeses, but the kinetics of death differed. Thus, for cheeses from pasteurized milk, *Enterobacteriaceae* counts were no longer detectable after 30 d ripening, while the decline was less pronounced in cheeses from raw milk. On the contrary, lactobacilli increased and enterecocci remained constant throughout ripening. Changes in microbial counts of cheeses throughout ripening are due to environmental conditions, such as a decrease in water activity (A_w), high concentration of salt, low pH and use of starters (Hernández-Jover et al. 1997).

Regarding biogenic amines, most gradually increased during ripening (Table 4). OC and DO were detected in only a few samples and at low levels (<1 mg/kg) and SE was not found in any sample. Changes in AG, SD and SM were not detected during ripening in either cheese. SM was the main polyamine, being $4{\cdot}05 \pm 1{\cdot}26$ and $3{\cdot}92 \pm 0{\cdot}93$ mg/kg, in cheeses from pasteurized and raw milk, respectively. Similar behaviour of microorganisms and similar amine profiles during ripening have been reported in cheeses made from cow milk (Schneller et al. 1997) and in Idiazábal and Tukum cheeses (made from ewe milk) (Pérez-Elortondo et al. 1993; Ordóñez et al. 1997; Durlo-Özkaya et al. 2000).

TY was the main aromatic biogenic amine present throughout ripening in raw milk cheeses. This result is

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Table 3. Counts [log (cfu/g)] of cheeses made from pasteurized and raw milks

Values are means ± SD for $n=6$

	PCA†		LAC		LAB		ENT		ENC	
Cheese	Pasteurized milk	Raw Milk	Pasteurized milk	Raw Milk	Pasteurized milk	Raw milk	Pasteurized milk	Raw milk	Pasteurized milk	Raw milk
1 d	$9{\cdot}76\pm0{\cdot}96^{aA}$	$9{\cdot}31\pm0{\cdot}91^{aA}$	$9{\cdot}72\pm0{\cdot}95^{aA}$	$9{\cdot}32\pm0{\cdot}91^{aA}$	$2{\cdot}62\pm0{\cdot}28^{aA}$	$5{\cdot}48\pm0{\cdot}54^{aB}$	$4{\cdot}69\pm0{\cdot}49^{aA}$	$6{\cdot}73\pm0{\cdot}65^{aB}$	$2{\cdot}57\pm0{\cdot}26^{aA}$	$5{\cdot}50\pm0{\cdot}54^{aB}$
14 d	$9{\cdot}68\pm0{\cdot}96^{aA}$	$9{\cdot}15\pm0{\cdot}90^{aA}$	$9{\cdot}40\pm0{\cdot}90^{aA}$	$9{\cdot}14\pm0{\cdot}89^{aA}$	$5{\cdot}49\pm0{\cdot}57^{bA}$	$7{\cdot}67\pm0{\cdot}78^{bB}$	$2{\cdot}45\pm0{\cdot}26^{bA}$	$4{\cdot}53\pm0{\cdot}77^{bB}$	$3{\cdot}39\pm0{\cdot}36^{aA}$	$6{\cdot}20\pm0{\cdot}74^{aB}$
30 d	$8{\cdot}43\pm0{\cdot}82^{aA}$	$8{\cdot}53\pm0{\cdot}85^{aA}$	$8{\cdot}04\pm0{\cdot}80^{aA}$	$8{\cdot}43\pm0{\cdot}84^{aA}$	$7{\cdot}37\pm0{\cdot}73^{cA}$	$8{\cdot}43\pm0{\cdot}91^{bB}$	nd^{cA}	$3{\cdot}77\pm0{\cdot}43^{bB}$	$2{\cdot}26\pm0{\cdot}62^{aA}$	$6{\cdot}59\pm0{\cdot}85^{aB}$
60 d	$7{\cdot}61\pm0{\cdot}76^{bA}$	$8{\cdot}08\pm0{\cdot}78^{aA}$	$7{\cdot}35\pm0{\cdot}73^{bA}$	$8{\cdot}03\pm0{\cdot}77^{aA}$	$7{\cdot}44\pm0{\cdot}76^{cA}$	$8{\cdot}23\pm0{\cdot}94^{bB}$	nd^{cA}	$3{\cdot}50\pm0{\cdot}76^{bB}$	$3{\cdot}61\pm0{\cdot}38^{aA}$	$6{\cdot}37\pm0{\cdot}79^{aB}$
90 d	$7{\cdot}18\pm0{\cdot}72^{bA}$	$7{\cdot}72\pm0{\cdot}69^{bA}$	$7{\cdot}15\pm0{\cdot}71^{bA}$	$7{\cdot}57\pm0{\cdot}71^{bA}$	$6{\cdot}93\pm0{\cdot}66^{cA}$	$7{\cdot}76\pm0{\cdot}77^{bA}$	nd^{cA}	$3{\cdot}16\pm0{\cdot}56^{bB}$	$3{\cdot}23\pm0{\cdot}54^{aA}$	$5{\cdot}98\pm0{\cdot}80^{aB}$

†PCA, aerobic mesophilic microorganisms; LAC, lactococci; LAB, lactobacilli; ENT, *Enterobacteriaceae*; ENC, =enterococci
nd, not detected for ENT, <100 (cfu/ml) or (cfu/g); for PCA, LAC, LAB and ENC <10 (cfu/ml) or (cfu/g)
[a]Values in columns for each microbial count with common superscripts were not significantly different ($P>0{\cdot}05$)
[A]Values in rows for each microbial count with common superscripts were not significantly different ($P>0{\cdot}05$)

Table 4. Biogenic amines contents (mg/Kg dry matter) of cheeses made from pasteurized and raw milks

Values are means (SD) for $n=6$

	Tyramine		Histamine		β-Phenylethylamine		Tryptamine		Cadaverine		Putrescine	
Cheese	Pasteurized	Raw milk	Pasteurized	Raw milk	Pasteurized	Raw milk	Pasteurized	Raw milk	Pasteurized	Raw milk	Pasteurized	Raw milk
1 d	$<0{\cdot}05^{aA}$	$4{\cdot}49^{aA}$ (5·88)	$<0{\cdot}05^{aA}$	$3{\cdot}05^{aA}$ (0·81)	$0{\cdot}73^{aA}$ (0·01)	$0{\cdot}80^{aA}$ (0·10)	$<0{\cdot}05^{aA}$	$1{\cdot}29^{aA}$ (1·32)	$1{\cdot}29^{aA}$ (0·01)	$53{\cdot}47^{aB}$ (14·98)	$0{\cdot}79^{aA}$ (0·01)	$10{\cdot}01^{aB}$ (6·97)
14 d	$0{\cdot}96^{aA}$ (0·01)	$29{\cdot}25^{bB}$ (43·46)	$1{\cdot}19^{aA}$ (1·81)	$3{\cdot}45^{aA}$ (0·05)	$1{\cdot}48^{aA}$ (1·02)	$2{\cdot}62^{aA}$ (0·90)	$2{\cdot}21^{aA}$ (0·01)	$3{\cdot}38^{aA}$ (0·79)	$1{\cdot}93^{aA}$ (0·01)	$72{\cdot}04^{bB}$ (6·74)	$3{\cdot}86^{aA}$ (5·09)	$33{\cdot}97^{bB}$ (9·14)
30 d	$2{\cdot}54^{aA}$ (0·31)	$93{\cdot}47^{cB}$ (86·26)	$2{\cdot}16^{aA}$ (0·31)	$5{\cdot}37^{aA}$ (3·09)	$4{\cdot}46^{aA}$ (0·60)	$6{\cdot}26^{bA}$ (6·89)	$5{\cdot}42^{aA}$ (0·01)	$7{\cdot}01^{bA}$ (0·34)	$5{\cdot}77^{aA}$ (1·81)	$87{\cdot}15^{bB}$ (15·72)	$7{\cdot}83^{bA}$ (0·01)	$42{\cdot}86^{bB}$ (7·15)
60 d	$7{\cdot}22^{bA}$ (4·31)	$245{\cdot}32^{dB}$ (162·50)	$3{\cdot}50^{aA}$ (1·92)	$27{\cdot}99^{bB}$ (22·81)	$7{\cdot}97^{bA}$ (1·13)	$19{\cdot}64^{cB}$ (4·70)	$6{\cdot}48^{bA}$ (3·40)	$9{\cdot}05^{bA}$ (2·06)	$15{\cdot}52^{bA}$ (21·42)	$177{\cdot}81^{cB}$ (22·49)	$8{\cdot}17^{bA}$ (10·10)	$74{\cdot}15^{cB}$ (17·03)
90 d	$10{\cdot}90^{bA}$ (14·75)	$324{\cdot}67^{eB}$ (145·99)	$6{\cdot}34^{bA}$ (3·30)	$43{\cdot}06^{cB}$ (36·47)	$8{\cdot}89^{bA}$ (3·88)	$27{\cdot}34^{dB}$ (8·00)	$7{\cdot}56^{bA}$ (3·58)	$12{\cdot}15^{cB}$ (4·54)	$32{\cdot}73^{cA}$ (34·53)	$196{\cdot}47^{cB}$ (32·01)	$14{\cdot}61^{cA}$ (14·25)	$86{\cdot}40^{cB}$ (29·83)

[a]Values in columns for each biogenic with common superscripts were not significantly different ($P>0{\cdot}05$)
[A]Values in rows for each biogenic amine with common superscripts were not significantly different ($P>0{\cdot}05$)

Table 5. Composition of cheeses made from pasteurized and raw milk

Values are means ± SD for $n=6$

	WSN/TN† (%)		Dry matter (g/100 g)		pH		Salt (g/100g)	
	Pasteurized milk	Raw milk	Pasteurized milk	Raw milk	Pasteurized milk	Raw milk	Pasteurized milk	Raw milk
1 d	$15{\cdot}57 \pm 0{\cdot}18^{AA}$	$15{\cdot}89 \pm 0{\cdot}19^{A}$	$48{\cdot}40 \pm 1{\cdot}35^{A}$	$47{\cdot}44 \pm 1{\cdot}73^{A}$	$4{\cdot}86 \pm 0{\cdot}07^{A}$	$4{\cdot}94 \pm 0{\cdot}06^{A}$	$1{\cdot}07 \pm 0{\cdot}16^{A}$	$1{\cdot}27 \pm 0{\cdot}25^{A}$
14 d	$16{\cdot}11 \pm 0{\cdot}33^{A}$	$17{\cdot}80 \pm 0{\cdot}39^{A}$	$54{\cdot}33 \pm 1{\cdot}25^{A}$	$52{\cdot}55 \pm 1{\cdot}44^{A}$	$4{\cdot}73 \pm 0{\cdot}07^{A}$	$4{\cdot}73 \pm 0{\cdot}04^{A}$	$1{\cdot}32 \pm 0{\cdot}13^{A}$	$1{\cdot}27 \pm 0{\cdot}19^{A}$
30 d	$21{\cdot}47 \pm 0{\cdot}37^{A}$	$24{\cdot}58 \pm 0{\cdot}41^{B}$	$60{\cdot}30 \pm 1{\cdot}58^{A}$	$58{\cdot}51 \pm 2{\cdot}15^{A}$	$4{\cdot}88 \pm 0{\cdot}07^{A}$	$5{\cdot}15 \pm 0{\cdot}06^{B}$	$1{\cdot}28 \pm 0{\cdot}35^{A}$	$1{\cdot}37 \pm 0{\cdot}43^{A}$
60 d	$22{\cdot}75 \pm 0{\cdot}34^{A}$	$26{\cdot}62 \pm 0{\cdot}29^{B}$	$66{\cdot}76 \pm 2{\cdot}03^{A}$	$64{\cdot}66 \pm 2{\cdot}76^{A}$	$5{\cdot}07 \pm 0{\cdot}05^{A}$	$5{\cdot}22 \pm 0{\cdot}07^{B}$	$1{\cdot}79 \pm 0{\cdot}08^{A}$	$1{\cdot}85 \pm 0{\cdot}15^{A}$
90 d	$23{\cdot}91 \pm 0{\cdot}15^{A}$	$27{\cdot}91 \pm 0{\cdot}22^{B}$	$72{\cdot}68 \pm 1{\cdot}51^{A}$	$71{\cdot}69 \pm 2{\cdot}86^{A}$	$5{\cdot}00 \pm 0{\cdot}06^{A}$	$5{\cdot}24 \pm 0{\cdot}12^{B}$	$2{\cdot}04 \pm 0{\cdot}25^{A}$	$2{\cdot}17 \pm 0{\cdot}31^{A}$

†WSN/TN, Water Soluble Nitrogen/Total Nitrogen
[A]Values in rows for each biogenic amine with common superscripts were not significantly different ($P>0{\cdot}05$)

consistent with previous reports in cow milk cheeses (Scheneller et al. 1997) and in Azeitâo cheese (made from ewe milk) (Pinho et al. 2001). The final amounts of TY in cheeses from raw milk were 30 times higher than in cheeses from pasteurized milk. This could be explained by the marked differences mainly in the enterococci counts, but also in those of lactobacilli (Table 3). According to Petridis & Steinhart (1996), the content of TY and PHE in cheese is associated with the number of enterococci. The accumulation of TY has also been related to non-starter lactic acid bacteria, mainly lactobacilli (Joosten & Northolt, 1987; Stratton et al. 1991; Novella-Rodríguez et al. 2002). The production of TY was also related to lactococci (Gónzalez de Llano et al. 1998; Bover-Cid & Holzapfel, 1999; Durlu-Özkaya et al. 1999). In the present work, lactococci counts were similar in both kinds of cheese reflecting the development of the starter, which was unable to produce biogenic amines (Novella-Rodríguez et al. 2002).

Several adverse effects for health could be linked to TY in cheese, such as hypertensive crisis and food-induced migraine in patients treated with mono amino oxidase inhibitors (MAOI). The intake of 6 mg of TY can produce these symptoms in patients receiving non-selective MAOI drugs (McCabe, 1986). For 30 g of cheese, a usual serving, only cheeses from raw milk would provide more than 6 mg of TY. When selective MAOI drugs are used, 50 mg of TY are necessary to produce adverse effects for health (Dollery et al. 1986) and none of the samples studied exceeded this amount.

The other biogenic amines found in the samples can be ordered according to their amount as follows: CA, PU, HI, PHE and TR, all of which showed higher contents in cheeses from raw milk (6–7 times greater) than in cheeses from pasteurized milk. The higher content of CA, PU and HI in batch from raw milk could be explained by the higher *Enterobacteriaceae* and lactobacilli counts found in that batch. These amines are commonly associated with *Enterobacteriaceae* (Stratton et al. 1991; Petridis & Steinhart, 1996; Sharaf et al. 1997) and they can also be produced by lactobacilli (Joosten & Northolt, 1987; Stratton et al. 1991; Novella-Rodríguez et al. 2002). Likewise, the relatively high number of lactobacilli in cheeses from pasteurized milk might explain why PU, CA and HI accumulated after 14 d ripening, even when *Enterobacteriaceae* were not detected.

HI has been often related with histaminic intoxication of food (Aygün et al. 1999). There are no legal regulations for HI in cheese, but none of the samples studied here exceeded the level regulated for fish.

Regarding the minor biogenic amines, PHE and TR, contents were also higher in cheeses from raw milk, with PHE and TR being three and two times higher than in cheeses from pasteurized milk, respectively. The production of PHE by enterococci in cheese has been related to tyrosine decarboxylase positive activity, since this enzyme can also use phenylalanine as substrate (Joosten & Northolt, 1987). In agreement with this, the cheeses with high levels of TY also exhibited high levels of PHE. However, the PHE values found in our study were lower than those reported by other researchers in ripened cheese made from cow milk (Schneller et al. 1997) and in Feta cheese (Valsamaki et al. 2000), but higher than those found in Idiazábal cheese (Ordoñez et al. 1997) and in cow cheese (Fernández-García et al. 2000).

The pH values varied throughout ripening according to the expected evolution in fermented cheese (Table 5). First, these values decreased as a result of starter bacteria metabolism and then they increased, values being slightly higher in cheeses from raw milk, which also showed the greatest formation of biogenic amines as Joosten (1988) also found. A greater proteolysis was observed in cheese made from raw milk (WSN/TN) (Table 5). Pasteurization can lead to physicochemical changes (López-Fandiño et al. 1996) such as slight denaturation of whey proteins and inactivation of some native milk proteases, both affecting proteolysis and availability of precursor amino acids of biogenic amines (Grappin & Beuvier, 1997). Moreover, an overall positive correlation was found between WSN/TN and the levels of the main biogenic amines, TY ($r=0{\cdot}6636$, $P<0{\cdot}05$), CA ($r=0{\cdot}6282$, $P<0{\cdot}05$) and PU ($r=0.0.6657$, $P<0{\cdot}05$). No statistical differences ($P>0{\cdot}05$) in salt were found between both kinds of cheese. Therefore, the differences in the biogenic amine contents between cheeses from pasteurized and raw milk can be mainly attributed

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to microorganisms and, to a lesser extent, to the degree of proteolysis.

Raw milk seems to be the main source of enterococci. However, the origin of these microorganisms in cheese elaborated from pasteurized milk is not clear enough since neither raw materials nor the evaluated critical points presented detectable amounts of these microorganisms.

In conclusion, the formation of biogenic amines can be strongly controlled if external contamination is avoided by strict hygienic manufacturing practices. To control the biogenic amine formation the quality of milk and hygiene during cheese manufacturing should also be optimized and standardized. Pasteurization of milk eliminates some of the bacteria that are the major cause of biogenic amine production in cheese, this being the main explanation for the lower amine contents in cheeses from pasteurized milk. In addition, other factors such as the degree of proteolysis can also play a critical role in amine biogenesis and should also be taken into account. However, it is also clear from our results that due to the high manipulation during cheese elaboration, the possibility of a post-pasteurization contamination with biogenic amine forming microorganisms (e.g. *Enterobacteriaceae* or enterococci) exists and consequently, the possibility of formation of biogenic amines. For this reason, the elaboration of the curd must be considered, like pasteurisation, a Critical Control Point during the implementation of HACCP for cheese manufacture. Perhaps, today, it is very difficult or impossible to obtain cheeses without any biogenic amine and maintaining all their sensory properties, but by using hygienic manufacturing practices it is possible to obtain cheeses with low or moderate levels of biogenic amines.

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