Comparison of Biogenic Amine Profile in Cheeses Manufactured from Fresh and Stored (4°C, 48 Hours) Raw Goat’s Milk

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ABSTRACT

In this study, the evolution of microbial counts, biogenic amine contents, and related parameters (pH, moisture, and proteolysis) in goat cheese made from fresh raw milk or raw milk stored for 48 h at 4°C was examined. In both cases the milk was nonpasteurized. This study was designed to evaluate the effect of milk quality on the profile of biogenic amines in relation to the evolution of the microbial population during cheese making. Cheese made from raw milk stored for 48 h at 4°C showed the highest microbial counts and biogenic amine levels. The storage of milk under refrigeration caused significant increases in the levels of some microbial and biogenic amines during ripening, but not initially. Tyramine was the main biogenic amine in the two cheeses tested, followed by cadaverine. However, the main differences in amine contents between batches were found for putrescine, histamine, and β-phenylethylamine, whose levels were more than twofold higher in samples from raw milk refrigerated for 48 h than in samples from fresh milk.

Raw goat’s milk is commonly used in some ripened cheeses produced in Mediterranean countries. These kinds of cheeses have a stronger flavor than those made from pasteurized milk, a feature greatly appreciated by consumers. Cheese made from raw goat’s milk is usually prepared in small batches and according to traditional practices. In most cases, this cheese is marketed as a “delicatessen” product, but in spite of its high market value, little information is available about its composition and hygienic quality. The quality of the primary ingredient, milk, is crucial for the quality of the cheese, and this factor increases in importance for cheese made from raw milk. In contrast to cheese made from pasteurized milk, cheese produced from raw milk is not subjected to any thermal treatment to reduce wild microflora involved in biogenic amine production. Hence, to ensure hygienic quality, the ripening period required for this cheese is longer than that for cheese made from pasteurized milk. For example, the current EU and Spanish regulations stipulate 2 months as the minimum ripening period for raw-milk cheese (5).

Biogenic amines are produced through the decarboxylation of certain amino acids catalyzed by specific decarboxylase enzymes of microorganisms (7, 15). The microbial quality of several foods has been correlated with biogenic amine accumulation. However, the ability of bacteria to decarboxylate different amino acids varies greatly. Thus, there is a correlation between histamine (HI), tyramine (TY), putrescine (PU), and cadaverine (CA) levels and lactobacilli (11, 22), between TY level and lactococci (6), and between TY and β-phenylethylamine (PHE) levels and enterococci (11, 13). Also, the formation of CA, PU, and HI in Enterobacteriaceae has been described (22, 25). Moreover, some bacterial strains exhibit proteolytic activity, which can enhance the accumulation of biogenic amines in cheese due to an increase in the number of free precursor amino acids (10, 20). Some of the strongest amine producers are bacteria that are closely associated with food spoilage. Therefore, the quantitative measurement of biogenic amines could be used as a chemical marker by which to establish the presence of microbiological contamination during food processing (14, 29, 30).

Several factors, such as pH and temperature, could affect the growth and activity of microorganisms during cheese making. Therefore, these factors could also play a key role in the accumulation of biogenic amines in cheese. In addition, the cheese-ripening process offers other favorable conditions for the formation of biogenic amines; for example, proteolysis gives rise to free amino acid precursors of amines, and an acidic environment favors the synthesis and activity of amino acid decarboxylases of microbial origin (3). The production of biogenic amines in cheese made from raw milk could be favored by the high microbial counts for this milk compared with those for pasteurized milk, as well as the compulsory longer ripening period.

The main biogenic amines in cheese are TY, CA, PU, HI, tryptamine (TR), and PHE. Some of these biogenic amines contribute to many human physiological functions, especially with regard to the nervous system and the control of blood pressure (7, 15). In addition to the use of biogenic amines as an index of hygienic quality, the measurement of their levels in food to assess the safety of a product is...
also recommended, since they may pose potential health risks. However, not all amines are equally toxic. TY, the main amine in cheese, has been related to food-induced migraines and hypertensive crises in patients receiving antidepressive treatment with monoamine oxidase inhibitor (MAOI) drugs, whereas HI has been reported to be the causative agent of scombroid poisoning (14, 15). Other biogenic amines in cheese, such as TR, PU, and CA, have been described as potentiators of these toxic effects because they compete for mechanisms involved in amine detoxification, such as monoamine oxidases, diamine oxidases, and hydroxymethyl transferases (26).

In this study, the effects of refrigerated storage of raw goat’s milk on biogenic amine contents and microbial counts throughout the cheese-making process were examined. We compared the changes occurring in goat cheese made from freshly obtained raw milk and those occurring in the same raw milk stored under refrigeration for 2 days before processing. A long ripening period was used in both cases, in compliance with the legal regulations for the production of raw-milk cheese, to check whether this practice is sufficient to control nonstarter microbial development and the accumulation of biogenic amines linked to milk storage. In addition to biogenic amine contents and microbial counts, pH, water content, and proteolysis-related parameters were also studied.

MATERIALS AND METHODS

Cheese manufacture. Two batches of goat cheese were manufactured in triplicate with fresh raw goat’s milk obtained from a local farm. Half of the milk was immediately used for cheese manufacture (batch R), and the other half was kept at 4°C for 48 h before being used (batch RS). Cheeses were made in the pilot plant of the Unit of Food Technology, Centre de Referència en Tecnologia d’Aiments, at the Autonomous University of Barcelona (Barcelona, Spain) by the usual procedure for cheese elaboration. A lactic starter (Lactococcus lactis subsp. lactis plus Lactococcus lactis subsp. cremoris; AM Larbus S.A., Barcelona, Spain) at 2% (vol/vol) was inoculated into milk. The decarboxylase activity of the starter culture was assayed in a previous study (17), and it was found not to produce biogenic amines under the in vitro conditions applied. Prior to the addition of the starter, milk (in the churns used for transport) was placed in a water bath at 50°C for 30 to 60 min to warm the milk to 32°C. Calf rennet (0.02%, vol/wt), containing 780 mg of chymosin per liter, and 35% (wt/wt) calcium chloride (0.025%, vol/wt) (Reniford-15/E, Limirsa, Barcelona, Spain) were also added. Coagulation occurred at 30 ± 1°C within approximately 45 min, and then curd was gently cut into 8- to 10-mm cubes. The curd was held for 5 min before it was stirred and further warmed to 32°C. When curd and whey reached the desired temperature, they were held for 15 min, and then the whey was immediately drained from the vat. Drained curds were molded into cylindrical holders and pressed in a pneumatic press at 0.7 kg/cm² for 48 h, lactococci were enumerated on M17 agar plates (Oxoid) after incubation at 30°C for 48 h, lactococci were enumerated on M17 agar plates (Oxoid) supplemented with lactose (Scharlau Microbiology, Barcelona, Spain) after incubation at 30°C for 48 h, lactobacilli were enumerated on Rogosa agar (Oxoid) after incubation at 30°C for 5 days in an atmosphere containing 10% CO₂, enterococci were enumerated on kanamycin aesculin azide agar (Oxoid) after incubation at 37°C for 48 h, Enterobacteriaceae were enumerated on violet red bile glucose agar (Biokar Diagnostics, Beuvais, France) after incubation with a double layer at 37°C for 24 h, Micrococcaceae were incubated on mannitol salt agar (Lio/lchrom, Roseto degli Abruzzi, TE, Italy) with 1% Tween 80 (Lio/lchrom) after incubation at 30°C for 48 h, and molds and yeasts were enumerated on rose bengal chloramphenicol agar (Scharlau) after incubation at 20°C for 5 days.

Physicochemical analysis. The total solid contents were analyzed with the use of International Dairy Federation standards (8). Total nitrogen (TN) and pH 4.6–soluble nitrogen (WSN) levels were obtained by a previously described procedure (12) and were then measured by the Kjeldahl method (9). The WSN/TN ratio was used as the proteolysis index. The pH was measured for a cheese/distilled water ratio of 1:1.

Statistical analysis. Microbial count and biogenic amine content data for samples were evaluated by the nonparametric Wilcoxon test, since data did not show a normal distribution. Linear regression analysis was used to determine relationships between ripening time, microbial counts, biogenic amine levels, proteolytic parameters, and pH. All statistical analyses were performed with the Statistical Package for Social Sciences, SPSS Version 9.0 for Windows (SPSS Inc., Chicago, Ill.). The results are expressed as average values ± standard deviations for three trials.

RESULTS AND DISCUSSION

Raw materials and curing. Neither microbes nor biogenic amines were found in any sample of CaCl₂, while in rennet they were detected at very low levels (aerobic mesophilic microorganisms were detected at <1 log CFU/ml). In spite of the low microbial counts, rennet showed an average TY level of 28.05 ± 1.25 mg/kg of and average HI, CA, and PU levels of 8 to 10 mg/kg. However, the actual contribution of rennet to biogenic amine content in the final formation of biogenic amines. Analyses were performed in duplicate.

Biogenic amines. Biogenic amine levels were measured by a previously described high-performance liquid chromatography procedure (19). This method is based on ion-pair partition chromatography involving a postcolumn reaction with o-phthalaldehyde and allows the resolution of 12 amines. These amines can be classified as (i) aromatic amines (TY, HI, PHE, TR, octopamine [OC], dopamine [DO], and serotonin [SE]), (ii) aliphatic diamines (PU and CA), and (iii) aliphatic polyamines (agmatine [AG], spermidine [SD], and spermine [SM]). Results corresponding to cheese samples were expressed as milligrams per kilogram (dry weight).

Microbiological analysis. Samples (10 g each) were placed in sterile Stomacher bags and homogenized for 2 min in 90 ml of buffered peptone water (pH 7.2 ± 0.2; Oxoid Ltd., Basingstoke, Hampshire, UK) with 1% Tween 80 (Liofilchem, Roseto degli Abruzzi, TE, Italy) with a 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 (Liofilchem) after incubation at 30°C for 48 h, lactococci were enumerated on M17 agar plates (Oxoid) supplemented with lactose (Scharlau Microbiology, Barcelona, Spain) after incubation at 30°C for 48 h, lactobacilli were enumerated on Rogosa agar (Oxoid) after incubation at 30°C for 5 days in an atmosphere containing 10% CO₂, enterococci were enumerated on kanamycin aesculin azide agar (Oxoid) after incubation at 37°C for 48 h, Enterobacteriaceae were enumerated on violet red bile glucose agar (Biokar Diagnostics, Beuvais, France) after incubation with a double layer at 37°C for 24 h, Micrococcaceae were incubated on mannitol salt agar (Liofilchem) after incubation at 30°C for 48 h, and molds and yeasts were enumerated on rose bengal chloramphenicol agar (Scharlau) after incubation at 20°C for 5 days.
product was very low (<0.6 μg/kg) because rennet is added to milk at a low concentration (20 ml of rennet per 100 liters of milk). In brine, aerobic mesophilic microorganisms and lactococci were detected at <5 log CFU/ml and Enterobacteriaceae were detected at <0.5 log CFU/ml. These microbial counts were similar to those reported by other authors for brine used in common cheese-making processes (4). Of the biogenic amines, only CA and PU were detected, at very low levels (<1 mg/kg). The milk additives were unimportant contributors to the microbial counts and biogenic amine contents in the initial mixture, and thus their influence on the final biogenic amine contents of cheese was minimal.

Storage clearly diminished the hygienic quality of milk, since microbial counts for both milk and curd samples from batch RS were statistically higher than those for samples from batch R, with the exception of lactobacillus and enterococcus counts, which were similar for milks and curds from both batches (Table 1). In contrast, biogenic amine contents for milk were low and quite similar between the two batches, with the natural polyamine SM always being the prevailing amine.

The heating of the milk from 4 to 32°C, which must be done prior to the addition of the starter, favored microbial growth and caused an increase in the differences in microbial counts (especially Enterobacteriaceae counts) between batches. However, the biogenic amine content of milk remained unchanged after the milk was heated to 32°C.

Most of the biogenic amines present in milk remained in the curd, since CA was the only amine detected in whey, and it was detected at very low levels (0.05 mg/kg). The differences in biogenic amine contents between batches became evident in the curds. The main difference was observed in CA levels, which were three times as high in batch RS. The occurrence of CA in cheeses has been related to Enterobacteriaceae (13, 22), and the increased presence of this amine in batch RS is consistent with the higher Enterobacteriaceae counts found for this batch. This relationship is also in agreement with the results of previous studies (22, 24). In addition to CA, the curds also showed higher levels of TY and PU than milk samples did. All three amines are commonly related to bacterial metabolism, and their formation in cheese as a result of Enterobacteriaceae and enterococcus amino acid decarboxylase activity has been reported (1, 14, 22).

Levels of the natural polyamines SD and SM were also higher in curd samples than in the corresponding milk samples. However, the slight differences between milk and curd samples are attributable to the concentration effect involved in curd elaboration rather than to microbial activity, because polyamines are not produced by microorganisms.

Cheese ripening. Initial counts of aerobic mesophilic microorganisms were similar for the two batches (9.31 ± 0.91 log CFU/g for batch R and 9.13 ± 0.60 log CFU/g for batch RS). These counts also showed similar profiles throughout the ripening process, decreasing from the initial level by 1 log unit over 90 days of ripening. Levels of

### TABLE 1. Microbial counts and concentrations of biogenic amines in milk or curd made from batches R and RS

<table>
<thead>
<tr>
<th>Sample</th>
<th>Microbial count</th>
<th>Concentration of biogenic amine (mg/kg dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Kin</td>
</tr>
<tr>
<td>Batch R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk at 4°C</td>
<td>4.46 ± 0.19</td>
<td>2.45 ± 0.10</td>
</tr>
<tr>
<td>Milk at 25°C</td>
<td>4.05 ± 0.06</td>
<td>2.10 ± 0.21</td>
</tr>
<tr>
<td>Curd</td>
<td>4.04 ± 0.07</td>
<td>2.07 ± 0.36</td>
</tr>
<tr>
<td>Batch RS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk at 4°C</td>
<td>5.82 ± 0.66</td>
<td>4.63 ± 0.01</td>
</tr>
<tr>
<td>Milk at 25°C</td>
<td>7.25 ± 0.35</td>
<td>7.00 ± 0.01</td>
</tr>
<tr>
<td>Curd</td>
<td>8.81 ± 0.50</td>
<td>7.83 ± 0.01</td>
</tr>
</tbody>
</table>

- **PCA**: aerobic mesophilic microorganisms; **LAC**: lactococci; **LAB**: lactobacilli; **ENC**: enterococci; **ENT**: Enterobacteriaceae; **TY**: tyramine; **CA**: cadaverine; **PU**: putrescine; **SM**: spermidine; **SPM**: spermine.
- **PC**: Log CFU/g for milk; Log CFU/g for curd.
lactococci and Enterobacteriaceae also decreased during ripening (Fig. 1), which is consistent with the behavior usually observed during this process. The lactococcus profiles during ripening were similar for cheeses from the two batches, but the counts of Enterobacteriaceae were somewhat higher for batch RS, although no statistical differences between counts for batch R and counts for batch RS were found. No differences between batches were found with respect to lactococcus and enterococcus counts, which both increased during ripening (Fig. 2). Levels of Micro-cocacceae and molds and yeasts decreased during ripening, and no differences were found between batches with respect to these levels (data not shown). Microbial counts during ripening followed trends similar to those for other raw-milk cheese production processes (18, 21, 24).

With regard to biogenic amines during ripening, OC and SE were not detected in any cheese sample, and DO was found only at low levels in some samples (<1 mg/kg). In contrast, TY, CA, PU, HI, PHE, TR, and AG were detected at high levels in most samples (Table 2). Overall, biogenic amine contents increased gradually during ripening and reached maximum values at the end of the process. The production of biogenic amines during cheese ripening has been extensively reported (11, 20, 24, 26, 27).

Polyamine contents did not differ between batches. Thus, for batches R and RS at the end of the ripening period (90 days), the SD concentrations were 4.95 ± 1.76 mg/kg and 6.03 ± 1.86 mg/kg, respectively, and the SM concentrations were 2.69 ± 0.69 mg/kg and 3.94 ± 1.04 mg/kg, respectively. These findings are consistent with the non-microbial origin of SD and SM (2). The concentrations of these polyamines are in the range reported for ripened cheeses, with the SD level generally being higher than SM level and with both levels being lower than those of the biogenic amines associated bacterial origins, TY, PU, CA, and HI (20, 24, 28).

CA was the prevailing amine in cheeses of after 1 day of ripening, but at the end of the ripening period the prevailing amine had become TY, followed by CA, PU, HI, PHE, and, at clearly lower levels, TR and AG. TY has been described as the main amine in raw-milk cheese (20, 23, 24). Throughout the ripening process, the levels of all biogenic amines except TR and AG were markedly higher for batch RS than for batch R. Although TY and CA were the major amines, the main differences in formation ratios between batches were for PU, HI, and PHE levels, which were more than twofold higher for batch RS than for batch R. The Wilcoxon test showed significant differences (P < 0.05) between batches with respect to TY, CA, PU, HI, and PHE levels. However, the differences between batch R and
batch RS with respect to microbial counts and biogenic amine levels were weaker than those found by Novella-Rodríguez et al. (18) when cheeses made from pasteurized milk were compared with cheeses made from raw milk by the same procedure used here.

After 90 days, AG concentrations in cheese samples from the two batches were similar; however, AG profiles differed considerably for the two batches during ripening. Thus, there was clear formation of AG (which increased from undetectable levels to almost 9 mg/kg [dry weight]) in batch R, while levels of this amine remained constant in batch RS throughout the ripening period. It is remarkable

that ripening did not increase the AG contents in samples in which levels of this amine were already present. The presence of AG in batch RS could be the result of microbial activity during milk storage, whereas in batch R, AG formation occurred during ripening. Very little information about the occurrence and formation of AG in cheese is available in the literature. In other foods such as meat, fish, and seafood, the formation of this amine follows a peak profile, and it has been related to the initial period of spoilage (1, 32), in which Enterobacteriaceae play a key role.

During ripening, the rate of the release of free amino acids usually increases, and thus the formation of biogenic amines may be favored. The proteolysis index was slightly higher for batch RS than for batch R, which indicates that a larger amount of free amino acids (Table 3) is available to decarboxylate to biogenic amines. The pHs of samples from batch RS were only slightly higher than those of samples from batch R at the beginning of the ripening period (Table 3), but throughout the process the differences between batches were not statistically significant.

Current Spanish and EU regulations stipulate that a ripening period of at least 2 months is required for raw-milk cheeses, although longer periods, like the 90-day period of the present work, are quite commonly used. However, 3 months of ripening was not long enough to compensate for the high nonstarter microbial load in stored milk, which was kept at 4°C for 48 h (batch RS), compared with that initially

### TABLE 2. Biogenic amine concentrations in cheese samples taken from batches R and RS during ripening

<table>
<thead>
<tr>
<th>Ripening time (days)</th>
<th>TY</th>
<th>CA</th>
<th>PU</th>
<th>HI</th>
<th>AG</th>
<th>PHE</th>
<th>TR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.49 (5.88) A</td>
<td>53.47 (14.98) A</td>
<td>10.01 (6.97) A</td>
<td>3.05 (0.81) A</td>
<td>nd A</td>
<td>0.80 (0.10) A</td>
<td>1.29 A</td>
</tr>
<tr>
<td>14</td>
<td>29.25 (72.04) A</td>
<td>33.97 (4.46) A</td>
<td>3.45 (0.05) A</td>
<td>1.80 (0.10) A</td>
<td>0.80 (0.10) A</td>
<td>2.62 (3.38) A</td>
<td></td>
</tr>
<tr>
<td>Batch RS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.70 (0.58) B</td>
<td>61.99 (10.15) A</td>
<td>15.38 (5.88) A</td>
<td>3.05 (0.05) A</td>
<td>0.63 (0.06) A</td>
<td>0.80 (0.06) A</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>53.15 (88.48) B</td>
<td>74.78 (21.57) B</td>
<td>7.63 (3.48) B</td>
<td>3.45 (0.34) B</td>
<td>2.62 (3.38) A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>141.16 (135.01) A</td>
<td>107.26 (36.94) B</td>
<td>11.93 (5.14) B</td>
<td>11.30 (1.23) A</td>
<td>7.62 (3.48) B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>329.17 (269.77) A</td>
<td>158.61 (19.98) B</td>
<td>52.45 (5.14) B</td>
<td>11.93 (1.23) A</td>
<td>43.85 (3.48) B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>427.83 (314.40) A</td>
<td>174.66 (19.98) B</td>
<td>83.29 (5.14) B</td>
<td>11.93 (1.23) A</td>
<td>92.24 (19.98) A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*TY, tyramine; CA, cadaverine; PU, putrescine; HI, histamine; AG, agmatine; PHE, β-phenylethylamine; TR, tryptamine; ND, not detected.

*Mean (standard deviation). Values for the same biogenic amine at the same sampling point with the same letter are not statistically different (P > 0.05) in batch R and in batch RS.

### TABLE 3. Proteolysis (water-soluble nitrogen [WSN] as a percentage of total nitrogen) and pH values for cheese samples taken from batches R and RS during ripening

<table>
<thead>
<tr>
<th>Ripening time (days)</th>
<th>% WSN</th>
<th>pH</th>
<th>% WSN</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch R</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15.89 (0.19)</td>
<td>4.94 (0.06)</td>
<td>14.95 (0.09)</td>
<td>5.18 (0.08)</td>
</tr>
<tr>
<td>14</td>
<td>17.80 (0.39)</td>
<td>4.73 (0.04)</td>
<td>21.16 (0.45)</td>
<td>4.86 (0.11)</td>
</tr>
<tr>
<td>30</td>
<td>24.58 (0.41)</td>
<td>5.15 (0.06)</td>
<td>26.35 (0.26)</td>
<td>5.04 (0.11)</td>
</tr>
<tr>
<td>60</td>
<td>26.62 (0.29)</td>
<td>5.22 (0.07)</td>
<td>26.23 (0.08)</td>
<td>5.32 (0.13)</td>
</tr>
<tr>
<td>90</td>
<td>27.91 (0.22)</td>
<td>5.24 (0.12)</td>
<td>28.24 (0.08)</td>
<td>5.36 (0.15)</td>
</tr>
</tbody>
</table>

*Values presented are means with standard deviations in parentheses.
observed for fresh milk (batch R). Given that levels of biogenic amines increased during ripening as a result of an accumulative effect, it is difficult to explain the differences between batches during this period.

Differences between the two batches with respect to biogenic amine contents were much greater for cheese samples than for initial milk samples, indicating the effect of milk quality during ripening. For instance, a 2-log difference in Enterobacteriaceae counts in initial milk samples led to a 25% increase in the TY level, a 50% increase in the CA level, and a 100% increase in the HI levels in the final product after 90 days. Cold storage of raw milk prior to cheese making allowed microbial growth and increased the probability of the survival of amine-forming bacteria during ripening, resulting in both higher microbial counts and a wider variety of bacterial strains. However, the more extensive formation of biogenic amines in batch RS may be the result of both the increased Enterobacteriaceae counts and the slightly higher degree of proteolysis in this batch. Moreover, it should be taken into account that the formation of biogenic amines is influenced by other factors, such as the starter culture used, the length of the ripening period, and the ability of microorganism to produce amines, which differs not only between microorganisms from distinct species but also between strains.

The accumulation of biogenic amines in cheese and in all foods should be avoided because of the adverse effects of these compounds on human health. Thus, 6 mg is the maximum tolerable dietary intake of TY for patients receiving nonselective MAOI drugs to prevent headaches or hypertensive crises, and 50 mg is the maximum allowable intake when selective MAOI drugs are used (16). If one considers 30 g of cheese a standard serving, both batches could pose potential health hazards after 60 days of ripening.

A maximum HI level of 20 mg/kg has been proposed for HI-intolerant individuals (31). In the present study, we found HI levels of >20 mg/kg in both batches after 60 days of ripening. However, levels of both amines were much higher in batch RS. Therefore, the consumption of cheeses made from stored milk (batch RS) is more likely to produce adverse effects.

In summary, the hygienic quality of raw goat's milk is crucial to ensure a low biogenic amine content in ripened cheese made from that milk, since regardless of the ability of the starter to form amines, the nonstarter bacteria contribute significantly to the production of these amines during the manufacture of cheese. Therefore, we conclude that the use of raw milk of high hygienic quality minimizes the accumulation of biogenic amines in this kind of cheese.

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