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Title: Effect of milk pasteurization and of ripening in a cave on biogenic amines content and sensory properties of a pecorino cheese

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Abstract: Cheesemaking trials were carried out with ewes' milk to evaluate the influence of pasteurization and of ripening in a cave on cheeses' biogenic amines (BA) content and on their sensory properties. At the end of the ripening period both factors influenced significantly the BA content with higher BA concentrations (on average more than 1500 mg kg-1 total and 850 mg kg-1 of tyramine) in cheeses manufactured with raw milk and partly ripened in a cave. Milk pasteurization effectively limited BA formation both qualitatively and quantitatively, but still allowed the accumulation of notable BA levels in cave ripened cheeses. Thus, milk pasteurization seems not sufficient to guarantee low BA levels in cheeses, particularly when the cheesemaking process employs unconventional ripening conditions. Discriminatory sensory testing showed that the different types of experimental cheeses had detectable sensory differences although descriptive sensory analysis highlighted few statistically significant differences, mainly due to the effect of the ripening conditions on some texture characteristics and on the aroma intensity.

# Effect of milk pasteurization and of ripening in a cave on biogenic amines content and sensory properties of a pecorino cheese

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**ABSTRACT**

Cheesemaking trials were carried out with ewes' milk to evaluate the influence of pasteurization and of ripening in a cave on cheeses' biogenic amines (BA) content and on their sensory properties. At the end of the ripening period both factors influenced significantly the BA content with higher BA concentrations (on average more than 1500 mg $kg^{-1}$ total and 850 mg $kg^{-1}$ of tyramine) in cheeses manufactured with raw milk and partly ripened in a cave. Milk pasteurization effectively limited BA formation both qualitatively and quantitatively, but still allowed the accumulation of notable BA levels in cave ripened cheeses. Thus, milk pasteurization seems not sufficient to guarantee low BA levels in cheeses, particularly when the cheesemaking process employs unconventional ripening conditions. Discriminatory sensory testing showed that the different types of experimental cheeses had detectable sensory differences although descriptive sensory analysis highlighted few statistically significant differences, mainly due to the effect of the ripening conditions on some texture characteristics and on the aroma intensity.

## 1. INTRODUCTION

The presence of biogenic amines (BA) in foods is mainly the result of amino acid decarboxylation by microbial enzymes. As such BA are naturally found in many foods, especially fermented ones, and their presence in cheeses is well known although with levels varying greatly in different types of cheese.

High levels of BA in food can have negative effects on consumers' health due to their toxicity. Histamine (HI), tyramine (TY), putrescine (PUT), cadaverine (CAD) and 2-phenylethylamine (2PHN) are the most common BA in food. Among these HI causes “scombroid fish poisoning”, while TY is responsible for the so called “cheese reaction”; other BA mostly have a potentiating effect (Silla-Santos, 1996). Therefore, it is desirable to limit BA accumulation in all foods including cheese.

The formation and accumulation of BA in cheese may be related to various amino acid decarboxylating microorganisms. Lactic acid bacteria, that have an important role in cheese production, can form HI, PUT and TY (Loizzo et al., 2013; Roig-Sagués, Molina, & Hernández-Herrero, 2002). *Enterobacteriaceae* have been mainly associated with HI, CAD and PUT formation (Bover-Cid & Holzapfel, 1999; ten Brink, Damink, Joosten, & Huis in 't Veld, 1990), while enterococci are known to produce PUT and TY (Ladero et al., 2012). It is difficult to correlate microbial counts with the amount of BA in cheeses because the decarboxylating activity of microorganisms is in most cases a strain related characteristic, and both the types and the quantities of BA produced differ widely among strains of the same species (EFSA, 2011).

Milk pasteurization is the most common treatment employed in cheesemaking to reduce pathogenic or contaminant microorganisms. Therefore, its effect on BA accumulation in cheeses has been previously studied and it is generally considered a useful tool to reduce BA levels (Novella-Rodríguez, Veciana-Nogués, Roig-Sagués, Trujillo-Mesa, & Vidal-Carou, 2004). Indeed, several data show a limiting action of pasteurization on BA production (Fernández, Linares, Del Rio, Ladero, & Alvarez 2007; Novella-Rodríguez, Veciana-Nogués, Izquierdo-Pulido, & Vidal-Carou, 2003; Novella-Rodríguez et al., 2004; Pattono, Bottero, Civera, Grassi, & Turi, 2002). However, Martuscelli et al. (2005) found no significant difference in total BA in an experimental “pecorino” cheese made with raw milk without starter and the same kind of cheese made with pasteurized milk and a starter culture. However, in experimental cheeses manufactured with cows' and ewes' milk, Lanciotti et al. (2007) on the contrary reported a lower BA content in raw milk cheese samples than in samples produced with milk subjected to thermal treatment.

The ripening process also influences BA levels in cheeses, since the proteolysis that takes place during this period increases the availability of amino acids, which can then undergo decarboxylation mediated by microbial enzymes (Novella-Rodríguez et al., 2003). Many studies on the evolution of the BA profile during the ripening of cheese have shown that BA content increases during this period (Fernández et al., 2007; Forzale et al., 2011a; Galgano et al., 2001; Komprda et al., 2008; Lanciotti et al., 2007; Martuscelli et al., 2005; Novella-Rodríguez et al., 2003; Novella-Rodríguez, Veciana-Nogués, Trujillo-Mesa, & Vidal-Carou, 2002; Pinho et al., 2004).

Few studies have been carried out on the effect of particular ripening conditions on the formation of BA in cheese. Mascaro et al. (2010) reported much higher total BA concentrations for cheeses ripened in a “fossa”, that is a traditional pit dug in volcanic rock (tuff), compared to control cheeses. However, unconventional ripening conditions, like ripening in a pit, in vessels buried in sand or soil (Kamber & Terzi, 2007) or in caves (Nuñez, 1978) have been traditionally employed to manufacture cheeses with peculiar organoleptic characteristics.

Following a previous study where high levels of BA were found in cave-ripened cheeses (Torracca, Nuvoloni, Ducci, Bacci, & Pedonese, 2015), the aim of this study was to assess the effect of milk pasteurization and of ripening in a cave (“grotto”) primarily on BA content of cheese and secondarily on its sensory properties.

## 2. MATERIALS AND METHODS

### 2.1 Cheesemaking trials

Three cheesemaking trials were carried out in a cheesemaking factory in the Province of Pisa in 3 different weeks in the month of May. The experimental cheeses were manufactured with ewe's milk using a commercial starter culture of non amine producing *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* (Lyofast MO 0.31, Sacco s.r.l., Cadorago, Como, Italy), as described for Type 4 cheeses in Torracca et al. (2015). In each trial, the same ewes' milk was used to manufacture 2 batches of cheese, each using 1,500 L of milk, one with pasteurized (70 °C, 40 seconds) and one with raw milk. Both types of cheese were ripened in 2 different ways: entirely in the ripening room of the factory (temperature: 7°C, relative humidity: 92%) or partly in the ripening room (2 months) and partly (2 months, from July to September) in a tuff cave (temperature: approximately 13 - 14 °C in winter, 17 - 18 °C in summer; relative humidity higher than 90%) in the province of Pisa, after being covered in straw. Thus, the manufactured types of cheeses differed for 2 factors: milk pasteurization and ripening conditions.

### 2.2 Cheese samples

Two samples of curd were collected for each cheesemaking trial, one manufactured with pasteurized milk and one with raw milk. For each cheesemaking trial, 6 cheese samples were collected after 2 months of ripening: 3 made with pasteurized milk and 3 with raw milk; and 12 cheese samples were collected after 4 months of ripening: 3 for each of the 4 types of cheese: made with pasteurized milk and ripened in the factory (PF) or ripened partly in a cave (PC), and made with raw milk and ripened in the factory (RF) or ripened partly in a cave (RC).

### 2.3 BA quantification

For all cheese samples the content of 8 BA, namely 2PHN, CAD, HI, PUT, spermidine (SPD), spermine (SPM), tryptamine (TRN), and TY, was quantified by HPLC analysis, using

1,7-diaminoheptane as an internal standard, dansyl-chloride for precolumn derivatization, a RP Gemini C18 column (250 mm x 4.60 mm, 5 µm) (Phenomenex, Torrance, CA, U.S.A) and a Jasco HPLC apparatus (Jasco Corporation, Tokyo, Japan). BA extraction, derivatization, and HPLC analyses were performed following the procedure described by Innocente, Biasutti, Padovese, & Moret (2007) with some modifications, as detailed in Torracca et al. (2015).

**2.4 Microbiological analysis**

Microbiological analysis was carried out to evaluate the presence of potentially decarboxylase-positive microorganisms. For each sample, 10 g were aseptically removed and homogenized with 90 mL of 2% (w/v) sterile sodium citrate solution using a 400 Circulator stomacher (PBI International, Milan, Italy). Dilutions were prepared with the same diluent and were used for standard plate enumeration. *Enterobacteriaceae* were determined on Violet Red Bile Glucose Agar (0.1 mL on spread plates) after incubation at 37 °C for 24 h; enterococci were enumerated on Kanamycin Aesculin Azide Agar base with Kanamycin Selective supplement (0.1 mL on spread plates) after 48 h of incubation at 42 °C, and lactobacilli were determined on MRS Agar (1 mL on pour plates) after incubation at 37 °C for 72 h under anaerobic conditions. All culture media and supplements were purchased from Oxoid Ltd. (Basingstoke, UK).

**2.5 Sensory analysis**

The samples collected after 4 months of ripening (end of the ripening process) were analyzed with discrimination and descriptive sensory techniques to assess the presence and the nature of sensory differences among the different types of samples. All samples were allowed to reach room temperature and codified anonymously with a 3 digit random number in cubes of approximately 1 $cm^3$ size and served following a balanced design (Macfie, Bratchell, Greenhoff, & Vallis, 1989). Unsalted crackers and water were available for mouth rinsing between samples. A triangle test was carried out to assess the presence of a detectable difference between samples that differed for only one of the studied parameters: pasteurization or ripening conditions. Thus, 4 triangle test comparisons were made: PF-PC, RF-RC, PF-RF, PC-RC and sixteen semi-trained and 8 trained panelists were involved in 6 sessions. For the quantitative descriptive analysis (QDA) a panel was formed with 8 trained panelists (3 men and 5 women; age range 26 – 55 years), chosen among the staff of the Department of Veterinary Sciences of Pisa University and trained following ISO 8586 (2012). Four training sessions were carried out on the quantification of sensory attributes in cheese. In 5 subsequent sessions, each panelist tasted 4 samples of each type of cheese (PF, PC, RF, RC). Nineteen sensory characteristics were considered, 7 related to aroma, 8 to flavour and 4 to texture. The 7 aroma characteristics were: aroma intensity, defined as the set of aromas commonly associated with ripened cheese; ewe's milk; animal/stable; butter; cooked milk; nutty; “acidity feel”, defined as a fermented lactic-acid aroma. The 8 flavour characteristics were: ewe's milk; bitter; sweet; piquant; animal/stable; nutty; butter; “acidity feel”, defined as a fermented lactic-acid aftertaste. The 4 texture characteristics were: hardness; granularity; fracturability; fatness. Sensorial analysis was carried out following ISO 4121 (2003) and using a 10 cm long unstructured scale.

**2.6 Statistical analysis**

All statistical analyses were performed with the software R v.3.0.2 and differences were considered significant if associated with a $P < 0.05$.

Results from microbial counts were previously converted in log cfu $g^{-1}$. For curd samples and 2 months cheese samples the statistical significance of differences, between pasteurized milk and raw milk cheese samples, in single and total amines and in microbial counts was tested with the *t* test. For 4 months samples a two-way ANOVA test, followed by Tukey HSD *post-hoc* comparisons, was performed using milk type (pasteurized and raw) and ripening conditions (in the factory or partly in a cave) as factors, to assess the significance of differences in single and total amines content, in QDA scores and in microbial counts among the different types of cheese.

For the sensory triangle test, the significance level of the number of correct answers was calculated using the binomial distribution.

## 3. RESULTS

### 3.1 BA quantification

The results regarding the BA content of cheese samples after 2 months of ripening in the factory are shown in Table 1. TY was the most abundant amine, followed by PUT, which was present in significant amounts, but only in cheese samples made with raw milk. 2PHN and TRN were detected in small amounts, CAD was detected only in raw milk cheese samples, while HI, SPD and SPM were never detected. The average total content of BA at 2 months of ripening was higher in raw milk cheese samples (158 mg $kg^{-1}$) than in pasteurized milk cheese samples (87 mg $kg^{-1}$), although the difference was not statistically significant.

The results regarding samples at the end of the ripening period (4 months) are detailed in Table 2. TY was again the most abundant amine, followed by PUT, 2PHN, TRN and CAD. HI and SPM were detected only in RC samples. SPD was never detected. The relative presence of the single amines was thus similar in 2 months and 4 months samples, but in general BA concentrations in 4 months samples were notably higher.

The highest total average BA content after 4 months was found in RC cheeses (1596 mg $kg^{-1}$), which also had the highest average TY concentration (871 mg $kg^{-1}$). Specifically, 6 of the RC samples had a TY concentration higher than 1,000 mg $kg^{-1}$ and among these 5 had a total BA concentration higher than 2,000 mg $kg^{-1}$. HI was detected only in 6 RC samples (maximum concentration: 52 mg $kg^{-1}$). On the contrary, only 1 PC sample ripened in a cave had a total BA concentration higher than 1,000 mg $kg^{-1}$ (1090 mg $kg^{-1}$) and it also had the highest TY concentration (810 mg $kg^{-1}$) among its type of samples.

Cheeses ripened entirely in the factory had lower BA concentrations and all these samples had a total BA concentration lower than 1,000 mg $kg^{-1}$ with a maximum level of 457 mg $kg^{-1}$ in a RF sample.

In fact, statistical analysis showed that both studied factors (milk pasteurization and ripening conditions), as well as their interaction, had a significant effect on TRN, 2PHN, PUT, HI, TY and total BA concentrations. Indeed, RC "pecorino" cheeses had a significantly higher concentration of TY, TRN, 2PHN, PUT, HI and of total BA content compared to the other 3 types of cheeses and they also had a significantly higher concentration of CAD compared to the 2 types of factory-ripened cheeses.

**3.2 Microbiological analysis**

In pasteurized milk curd samples no *Enterobacteriaceae* or enterococci were detected, while average lactobacilli load was 0.65 log cfu $g^{-1}$. Microbial counts for raw milk curd samples were 1.33 log cfu $g^{-1}$, 2.84 log cfu $g^{-1}$, and 3.44 log cfu $g^{-1}$, respectively for *Enterobacteriaceae*, enterococci and lactobacilli. The differences in enterococci and lactobacilli counts between pasteurized and raw milk curd samples were significant.

Microbial counts for 2 months and 4 months samples are shown in Table 3. After 2 months of ripening, microbial counts of samples of cheese made with raw milk were significantly higher than those of samples made with pasteurized milk. Regarding the results at the end of the ripening period, no statistically significant difference was found for *Enterobacteriaceae*, which were detected only in 3 samples, all cave-ripened (2 PC and 1 RC samples). Milk pasteurization had a significant effect on enterococci counts with higher microbial counts in raw milk cheese samples at the end of the ripening period. For lactobacilli, after 4 months of ripening PF samples had significantly lower microbial loads compared to the other 3 types of samples.

**3.3 Sensory analysis**

The results of the sensory discrimination test (triangle test) are detailed in Table 4. For all comparisons the number of different samples correctly identified was statistically significant. This result shows that the different types of experimental cheeses could be distinguished one from the other on the basis of their sensory characteristics. The QDA results are shown in Figure 1. The effect of pasteurization resulted statistically significant for the "acidity feel" characteristic of both aroma and flavour and for the flavours piquant and animal, which were all higher in the raw milk samples. The ripening conditions had a significant effect on aroma intensity and, among the texture characteristics, on hardness and fragility, which were all higher in cave-ripened samples.

## 4. DISCUSSION

In this study TY was always the most abundant amine, followed by PUT, 2PHN, TRN and CAD. HI and SPM were only detected in 4 months RC samples, while SPD was never detected. These data on the relative presence of amines are in accordance with previous studies on ewe's milk cheeses (Forzale, Nuvoloni, Pedonese, D'Ascenzi, & Giorgi, 2011b; Lanciotti et al., 2007; Mascaro et al., 2010; Pinho et al., 2004; Schirone, Tofalo, Mazzone, Corsetti, & Suzzi, 2011; Torracca et al., 2015). Samples analyzed after 2 months of ripening showed BA levels notably lower than those of cheeses at the end of the ripening period (4 months). Indeed, it is well known that the length of the ripening process is an important factor allowing BA accumulation in cheeses (Fernández et al., 2007; Komprda et al., 2008; Novella-Rodríguez et al., 2003; Pinho et al., 2004).

At the end of the ripening period, all samples had an average TY concentration in or above the range of 100 - 800 mg $kg^{-1}$, which is a safe level for TY proposed by some authors (ten Brink et al., 1990). In particular, 6 RC samples out of 9 total RC samples had more than 1,000 mg $kg^{-1}$ of TY. In both types of cave-ripened samples (PC and RC) the levels of 2PHN also exceeded a safe level as proposed by the same authors (30 mg $kg^{-1}$ ) (ten Brink et al., 1990). Total BA concentrations higher than 1000 mg $kg^{-1}$ have been previously reported for ewe's milk ripened cheeses (Martuscelli et al., 2005; Mascaro et al., 2010; Schirone et al., 2011; Torracca et al., 2015). It is noteworthy that in our study only RC samples had an average total BA content above 1,000 mg $kg^{-1}$ (1596 mg $kg^{-1}$) thus exceeding a safe level as proposed by Silla-Santos (1996).

Despite the high levels of BA, HI, the BA mainly responsible of intoxication cases, was detected in very limited amounts in RC samples or not detected at all in all the other samples. These data are in agreement with those reported for other Italian ewes' milk cheeses (Forzale et al., 2011a; Lanciotti et al., 2007; Mascaro et al., 2010; Schirone et al., 2011; Torracca et al., 2015), although higher levels have been found in ewes' milk cheeses both in Italy (Martuscelli  et al., 2005) and in other countries (Fernández-García, Tomillo, & Núñez, 1999; Pinho, Ferreira, Mendes, Oliveira, & Ferreira, 2001).

Regarding the effect of milk pasteurization on BA levels, pasteurized milk cheeses after 2 months of ripening already showed a lower total BA concentration, almost half of that of raw milk cheeses, although this difference did not prove to be statistically significant, probably due to the high variability within the same type of samples. At the end of the ripening period (4 months), the pasteurization factor proved to be significant for total BA concentration and for single amines, except for CAD, SPD and SPM, with BA levels in raw milk cheeses more than double those of the corresponding pasteurized milk ones. Thus, our data support the idea that pasteurization is an effective tool to reduce the contents of BA in cheese (Novella-Rodríguez et al., 2004). TY accounted for more than 80% of the total amines in pasteurized cheese samples (92.0% in PF samples and 81.9% in PC samples), while in raw milk samples TY represented at maximum the 65.4% of total amines. Similar data have been found in a previous study on ewe's milk cheeses produced in Tuscany (Torracca et al., 2015), therefore it seems that pasteurization also had an effect on the relative presence of each amine, maybe caused by a reduction of the variability of decarboxylase-positive microorganisms in milk. Moreover, the microbiological analysis confirms the efficacy of pasteurization in reducing the microbial loads in all types of samples, particularly for *Enterobacteriaceae* and enterococci, microbial groups often associated with the production of amines other than TY (Bover-Cid & Holzapfel, 1999; Ladero et al., 2012; Marino, Maifreni, Moret, & Rondinini, 2000; ten Brink et al., 1990).

High levels of BA in cheeses have often been associated with a low hygienic quality of milk (Andic, Genccelep, & Kose, 2010; Pintado et al., 2008). In our study *Enterobacteriaceae* counts were instead very limited and lower than those often reported in raw ewes' milk cheeses (Freitas & Malcata, 2000; Pinho et al., 2004; Pintado et al., 2008). Enterococci counts were also lower than those reported for Italian ewes' milk cheeses, while lactobacilli counts were in agreement with previously reported values (Schirone, Tofalo, Visciano, Corsetti, & Suzzi, 2012). In cheeses enterococci may be responsible for TY decarboxylation, especially considering that some authors report that this characteristic could be a species-level trait for *E. durans*, *E. faecium*, and *E. faecalis* (Ladero et al., 2012). Thus, the high levels of TY in our experimental cheeses could be at least partly due to enterococci. Indeed, the formation of TY and 2PHN in cheeses caused by enterococci has been reported by Joosten & Northolt (1987), although high bacterial counts (more than $10^7$ cfu $g^{-1}$) were necessary for TY to accumulate in significant amounts. In our cheeses enterococci counts were instead always lower than $10^5$ ufc $g^{-1}$. Lactobacilli counts were much higher ($10^6$- $10^7$ cfu $g^{-1}$) both in 2 months and 4 months cheese samples. It is known that some lactobacilli strains can have tyrosine-decarboxylating activity (Bover-Cid & Holzapfel, 1999; Lorencová et al., 2012; Roig-Sagués et al., 2002). This microbial group, due to its high counts, could therefore have played an important role in TY accumulation in our experimental cheeses.

As for the ripening conditions factor, it proved to be highly significant, with cave-ripened cheeses having higher BA levels (more than 3 times) compared to factory-ripened ones. Since environmental conditions affect the microbial populations in food, it is not surprising that the type of ripening has an effect on microbial counts and on BA levels. The environment inside a ripening cave is not as extreme as the one studied by Mascaro et al. (2010) inside a "fossa", where anaerobic conditions are established. None the less, the specific environmental conditions in the natural cave, such as the different relative humidity and temperature compared to the controlled factory environment, could have affected BA formation and accumulation.

Therefore, in cave-ripened cheeses, pasteurization alone, although limiting the BA content as previously noted, was not sufficient to contain BA formation. There was though an interaction between the pasteurization and the ripening conditions factors. Indeed, the highest BA content was found in RC cheeses.

As for the descriptive sensory analysis, it did not allow to find significant differences among the 4 types of experimental cheeses, except for one characteristic (“acidity feel” aroma higher in RC samples). Even though it is possible that a different selection of evaluated characteristics or the use of a different scale could have better highlighted the sensory differences among the 4 types of cheeses, it is also true that they were all similar products. Nevertheless, there was a difference and it was detectable, as the triangle test results showed. Indeed, the ripening conditions factor was significant for some texture characteristics and for the aroma intensity, with higher values in cave-ripened samples, which were thus perceived as “more ripened”. According to some authors (Gosetti, Mazzucco, Gianotti, Polati, & Gennaro, 2007) BA are also partly responsible for aroma and flavour characteristics, so much that each type of cheese can be associated with a specific BA profile that could be used for origin and authenticity evaluation. It is indeed possible that in our study the different BA content contributed to differentiate the various type of samples in relation to their sensory characteristics.

## 5. CONCLUSIONS

Our study confirms that technological aspects play a role in the BA accumulation in cheeses. In this regard, while milk pasteurization was a limiting factor, it was not sufficient to guarantee low amines' concentrations in the final product, since the length and type of the ripening process are also determining factors. On the other hand, these particular ripening processes are employed to manufacture cheeses with peculiar sensory properties recognized and appreciated by consumers. Moreover, while pasteurization is an operation easy to perform, in order to limit the occurrence of amine producing microorganisms, it is more difficult to intervene in other technological stages, such as the ripening in a traditional "*grotta*". A possible approach for the producers could be to monitor the environmental parameters of the ripening locations, intervening where possible to remove or reduce criticalities, such as excessive temperature leaps or an anomalous contamination of the site.

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**Table**

Table 1. Concentrations (average mg $kg^{-1}$ ± standard deviation) and percentage compositions of single and total biogenic amines in cheese samples after 2 months of ripening.[a]

| | Pasteurized milk | | Raw milk | |
|---|---|---|---|---|
| | Concentration (mean ± SD) | % of total | Concentration (mean ± SD) | % of total |
| Tryptamine | 6 ± 3[a] | 6.6 | 9 ± 4[a] | 57.8 |
| 2-phenylethylamine | 6 ± 5[a] | 6.4 | 2 ± 2[a] | 1.0 |
| Putrescine | ND[a] | -- | 55 ± 61[b] | 34.6 |
| Cadaverine | ND[a] | -- | 2 ± 2[b] | 1.3 |
| Tyramine | 75 ± 45[a] | 87.1 | 90 ± 52[a] | 57.3 |
| Total | 87 ± 45[a] | 100 | 158 ± 97[a] | 100 |

[a] Abbreviations are: ND, not detected. Histamine, spermidine and spermine were never detected. Different letters in the same row show statistically significant differences ($p < 0.05$).

**Table**

Table 2. Concentrations (average mg kg$^{-1}$ ± standard deviation) and percentage compositions of single and total biogenic amines in cheese samples at the end of the ripening period (4 months).[a]

| | P | | | | R | | | | Factors | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | F | | C | | F | | C | | P/R | F/C | P/R*F/C |
| | Concentration (mean ± SD) | % of total | Concentration (mean ± SD) | % of total | Concentration (mean ± SD) | % of total | Concentration (mean ± SD) | % of total | | | |
| Tryptamine | 3 ± 2[a] | 1.9 | 13 ± 11[a] | 2.3 | 14 ± 5[a] | 4.6 | 70 ± 47[b] | 4.4 | *** | *** | ** |
| 2-phenylethylamine | 10 ± 6[a] | 6.1 | 53 ± 54[a] | 9.2 | 8 ± 7[a] | 2.8 | 144 ± 108[a] | 9.0 | * | *** | * |
| Putrescine | ND[a] | -- | 25 ± 29[a] | 4.4 | 84 ± 47[a] | 27.2 | 442 ± 211[b] | 27.7 | *** | *** | *** |
| Cadaverine | < 1[a] | 0.1 | 13 ± 21[a,b] | 2.3 | < 1[a] | 0.1 | 29 ± 21[b] | 1.8 | NS | *** | NS |
| Histamine | ND[a] | -- | ND[a] | -- | ND[a] | -- | 29 ± 22[b] | 1.8 | *** | *** | *** |
| Tyramine | 149 ± 73[a] | 92.0 | 471 ± 271[a] | 81.9 | 201 ± 96[a] | 65.4 | 871 ± 448[b] | 54.6 | * | *** | * |
| Spermidine | ND | -- | ND | -- | ND | -- | ND | -- | NS | NS | NS |
| Spermine | ND[a] | | ND[a] | | ND[a] | | 11 ± 32[a] | 0.7 | NS | NS | NS |
| Total | 162 ± 78[a] | 100 | 574 ± 356[a] | 100 | 308 ± 134[a] | 100 | 1596 ± 828[b] | 100 | *** | *** | ** |

[a] Abbreviations are: P, pasteurized milk; R, raw milk; F, ripened in the factory; C, ripened partly in a cave; ND, not detected. NS, not statistically significant. Different letters in the same row show statistically significant differences ($p < 0.05$). *, p<0.05; **, p<0.01; ***, p<0.001.

**Table**

Table 2. Concentrations (average mg $kg^{-1}$ ± standard deviation) and percentage compositions of single and total biogenic amines in cheese samples at the end of the ripening period (4 months).[a]

| | P | | | | R | | | | Factors | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | F | | C | | F | | C | | P/R | F/C | P/R*F/C |
| | Concentration (mean ± SD) | % of total | Concentration (mean ± SD) | % of total | Concentration (mean ± SD) | % of total | Concentration (mean ± SD) | % of total | | | |
| Tryptamine | 3 ± 2[a] | 1.9 | 13 ± 11[a] | 2.3 | 14 ± 5[a] | 4.6 | 70 ± 47[b] | 4.4 | *** | *** | ** |
| 2-phenylethylamine | 10 ± 6[a] | 6.1 | 53 ± 54[a] | 9.2 | 8 ± 7[a] | 2.8 | 144 ± 108[a] | 9.0 | * | *** | * |
| Putrescine | ND[a] | -- | 25 ± 29[a] | 4.4 | 84 ± 47[a] | 27.2 | 442 ± 211[b] | 27.7 | *** | *** | *** |
| Cadaverine | < 1[a] | 0.1 | 13 ± 21[a,b] | 2.3 | < 1[a] | 0.1 | 29 ± 21[b] | 1.8 | NS | *** | NS |
| Histamine | ND[a] | -- | ND[a] | -- | ND[a] | -- | 29 ± 22[b] | 1.8 | *** | *** | *** |
| Tyramine | 149 ± 73[a] | 92.0 | 471 ± 271[a] | 81.9 | 201 ± 96[a] | 65.4 | 871 ± 448[b] | 54.6 | * | *** | * |
| Spermine | ND[a] | | ND[a] | | ND[a] | | 11 ± 32[a] | 0.7 | NS | NS | NS |
| Total | 162 ± 78[a] | 100 | 574 ± 356[a] | 100 | 308 ± 134[a] | 100 | 1596 ± 828[b] | 100 | *** | *** | ** |

[a] Abbreviations are: P, pasteurized milk; R, raw milk; F, ripened in the factory; C, ripened partly in a cave; ND, not detected. NS, not statistically significant. Spermidine was never detected. Different letters in the same row show statistically significant differences ($p < 0.05$). *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Table

Table 3. Viable counts (average log cfu $g^{-1}$ ± standard deviation) of *Enterobacteriaceae*, enterococci, and lactobacilli in cheese samples at 2 months and at the end of the ripening period (4 months).[a]

| | | | *Enterobacteriaceae* | Enterococci | Lactobacilli |
|---|---|---|---|---|---|
| 2 months | P | | 0.43 ± 0.86[a] | 2.55 ± 1.08[a] | 7.02 ± 0.52[a] |
| | R | | 1.58 ± 0.95[b] | 4.26 ± 0.61[b] | 7.48 ± 0.11[b] |
| | | | *Enterobacteriaceae* | Enterococci | Lactobacilli |
| 4 months | P | F | 0.00 ± 0.00[A] | 2.54 ± 0.79[A] | 6.04 ± 0.53[A] |
| | | C | 0.47 ± 0.96[A] | 2.34 ± 1.53[A] | 6.85 ± 0.44[B] |
| | R | F | 0.00 ± 0.00[A] | 4.48 ± 0.17[B] | 7.16 ± 0.08[B] |
| | | C | 0.19 ± 0.57[A] | 4.17 ± 0.16[B] | 7.19 ± 0.12[B] |
| | Factors | P/R | NS | *** | *** |
| | | F/C | NS | NS | ** |
| | | P/R*F/C | NS | NS | ** |

[a] Abbreviations are: P, pasteurized milk; R, raw milk; F, ripened in the factory; C, ripened partly in a cave. NS, not statistically significant. For 2 months samples different lowercase letters in the same column show statistically significant differences ($p < 0.05$). For 4 months samples different uppercase letters in the same column show statistically significant differences ($p < 0.05$). *, p<0.05; **, p<0.01; ***, p<0.001.

**Table**

Table 4. Triangle test results for discrimination sensory testing of the different types of experimental cheeses.[a]

| Comparison | Answers | Correct answers | % correct answers | Statistical analysis |
|---|---|---|---|---|
| PF - RF | 30 | 18 | 60.0 | ** |
| PC - RC | 58 | 35 | 60.3 | *** |
| PF - PC | 52 | 36 | 69.2 | *** |
| RF - RC | 52 | 30 | 57.7 | *** |

[a] Abbreviations are: PF, pasteurized milk cheese ripened in the factory; RF, raw milk cheese ripened in the factory; PC, pasteurized milk cheese ripened partly in a cave; RC, raw milk cheese ripened partly in a cave. **, $p<0.01$; ***, $p<0.001$.

**Figure**

Figure 1. Results of the quantitative descriptive sensory analysis of cheeses.[a]

[a] Abbreviations are: PF, pasteurized milk cheese ripened in the factory; RF, raw milk cheese ripened in the factory; PC, pasteurized milk cheese ripened partly in a cave; RC, raw milk cheese ripened partly in a cave. A-IN, aroma intensity; A-EM, ewe's milk aroma; A-AS, animal/stable aroma; A-BU, buttery aroma; A-CM, cooked milk aroma; A-NU, nutty aroma; A-AC, “acidity feel” aroma; F-EM, ewe's milk flavour; F-BU, buttery flavour; F-BI, bitter flavour; F-SW, sweet flavour; F-PI, piquant flavour; F-AS, animal/stable flavour; F-NU, nutty flavour; F-AC, “acidity feel” flavour; T-HA, hardness; T-FR, fracturability; T-GR, granularity; T-FA, fatness.

Figure
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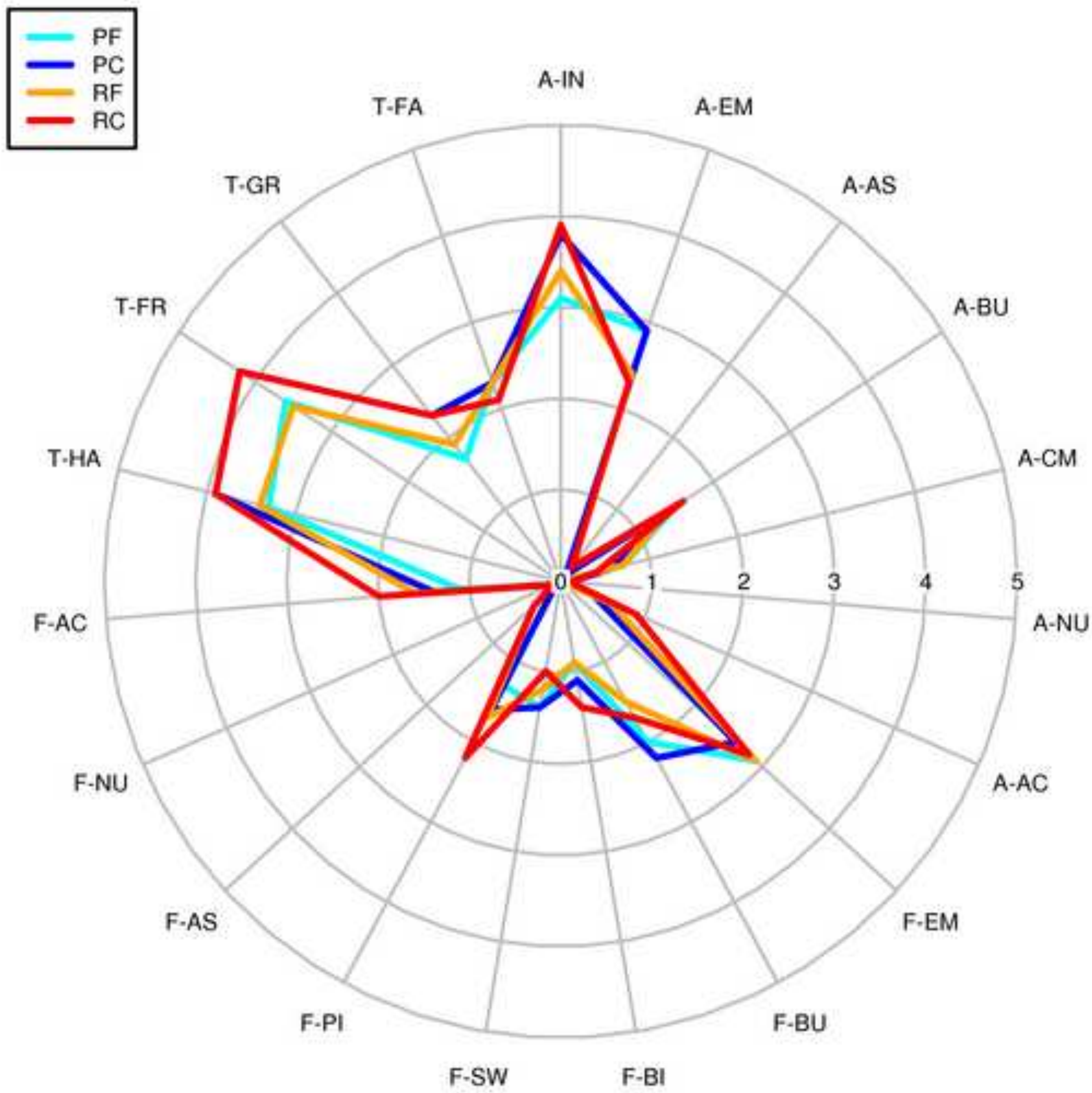

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