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Short communication

Effect of autochthonous starter cultures on the biogenic amine content of ewe's milk cheese throughout ripening

E. Renes [a], I. Diezhandino [a], D. Fernández [a], R.E. Ferrazza [b], M.E. Tornadijo [a, *], J.M. Fresno [a]

[a] Department of Food Hygiene and Technology, Faculty of Veterinary Science, University of Leon, 24071, León, Spain
[b] Department of Nutrition, Faculty of Nutrition, Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil

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ABSTRACT

Cheese is among the most commonly implicated foods associated with biogenic amines poisoning. The aim of this study was to evaluate the effects of the type of autochthonous starter culture and ripening time on the concentration of biogenic amines (histamine, tyramine, putrescine, cadaverine, tryptamine, β-phenylethylamine, spermine and spermidine) in cheeses made from pasteurized ewe's milk. 4 cheese batches were made, in duplicate, and ripened for 7 months. The biogenic amines of 40 cheeses were analysed by high performance liquid chromatography. The predominant biogenic amines determined at the end of the ripening time were phenylethylamine, spermine and tryptamine. Together, these accounted for 81% of the total of biogenic amines studied.

The type of starter culture used to make the ewe's cheese had a significant effect ($p < 0.001$) on the content of biogenic amines throughout ripening time. It was lower in the batches made with an autochthonous starter culture made up entirely of *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* or of the same in combination with *Lactobacillus plantarum*.

1. Introduction

Biogenic amines are organic, basic, nitrogenous compounds of low molecular weight, mainly formed by decarboxylation of amino acids (Komprda et al., 2007). They are involved in natural biological processes such as synaptic transmission, blood pressure control, allergic response and cellular growth control. Under normal conditions, the organism is able to degrade any biogenic amines ingested with food through the action of monoamine oxidases and diamine oxidases that detoxify these compounds (Broadley, 2010). Nonetheless, these mechanisms can be affected by various factors: genetic, physiological, those arising from eating foods with high levels of amines, or those caused by the consumption of certain specific inhibitors of monoamine oxidases (drugs, tobacco, and/or alcohol). Under these conditions, biogenic amines can have direct effects on human health (EFSA, 2011).

Currently, there is no legislation defining the limits of biogenic amines tolerance in fermented foodstuff. However, cheese is the food that reach the highest biogenic amine concentrations, and therefore, a more severe control should be exercised (Linares et al., 2012). Most of the Gram negative bacteria described as usual contaminants of milk are able to produce cadaverine, histamine or putrescine. In those cases, biogenic amines are the result of bad manufacturing practices, poor quality or insufficient hygienic conditions. However, the main biogenic amine producers in cheese are Gram positive bacteria, being lactic acid bacteria (LAB) the main histamine and tyramine producers (Linares et al., 2012). On the other hand, recent studies pointed out the importance of this bacterial group as putrescine producers (Ladero et al., 2011, 2012). In general, it is difficult to find a correlation between the presence of high concentrations of biogenic amines in cheeses with and increment of a specific group of LAB. This is due to the fact that the capability to produce amines is mostly related to strain rather than to specie (Novella-Rodríguez et al., 2002).

The typical aroma and taste characteristics of a cheese depend on various factors, lactic bacteria starters being the principal factor responsible for releasing specific flavour compounds. This fact has led to there being increasing concern on the part of cheesemakers with regard to the type of starter cultures they should use. In fact, craft or "artisanal" cheeses constitute a source of strains with physiological or biochemical properties of technological interest. These autochthonous lactic acid bacteria cultures may be applicable

* Corresponding author. Tel.: +34 987 293253; fax: +34 987 291284.
E-mail address: metorr@unileon.es (M.E. Tornadijo).

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at an industrial scale in the production of cheeses, contributing to guaranteeing that they are hygienic and healthy and to preserving the typical sensory characteristics of each variety (Hébert et al., 2000; Minervini et al., 2009). Moreover, the lactic acid bacteria that do not normally enter the composition of the starter (NSLAB) also have a marked influence upon the flavour, aroma and texture of cheeses and hence their quality (Sheedan et al., 2008). The choice of autochthonous LAB strains on the basis of their profile of enzyme activity offers a chance to have starters reproducing the typical characteristics of each variety. Nevertheless, some strains may also give rise to the liberation of compounds that in high concentrations may prove toxic for consumers, as is the case for biogenic amines. In the fact, during cheese ripening, there is a breaking down of caseins, which leads to an accumulation of free amino acids that can be transformed into biogenic amines by the decarboxylase activity of the microbiota present (Sihufe et al., 2010).

The aim of this work was to assess the effects of the type of autochthonous starter culture and the ripening time on the concentration of biogenic amines (histamine, tyramine, putrescine, cadaverine, tryptamine, β- phenylethylamine, spermine and spermidine) in a cheese made from pasteurized ewe's milk.

2. Materials and methods

2.1. Cheese manufacture and sampling

The strains used in the autochthonous starter cultures belonged to the collection of Food Technology Area of the University of Leon (Spain). They were characterized from a technological viewpoint (acidifying and proteolytic activities) by Herreros et al. (2003). The identity of the strains was confirmed by use genetic techniques (Sandoval et al., 2004).

Four batches of ewe's cheese were produced by duplicate (eight batches in total, forty cheeses) in accordance with the method described. Calcium chloride (0.2 g/L) and starter culture (1%) were added to pasteurized ewe's milk (72 °C for 15 s). Starter cultures used were as follows: Batch 1: the commercial culture CHOOZIT™ LYO MA 011 (*Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*); Batch 2: an autochthonous culture with *L. lactis* subsp. *lactis* (TAUL 1292) and *L. lactis* subsp. *cremoris* (TAUL 1239); Batch 3: an autochthonous culture with *L. lactis* subsp. *lactis* (TAUL 1292), *L. lactis* subsp. *cremoris* (TAUL 1239) and *Lactobacillus plantarum* (TAUL 1736); Batch 4: an autochthonous culture with *L. lactis* subsp. *lactis* (TAUL 1292), *L. lactis* subsp. *cremoris* (TAUL 1239) and *Enterococcus raffinosus* (TAUL 1351). After half an hour, 30 mL of liquid sheep's rennet (80% chymosin, 75 Rennet Units, Cuajos Caporal, Valladolid, Spain) was added per 100 L of milk, coagulation occurring in 40–45 min. The curds were then cut with special cutting tools for approximately 20 min until they reached a rice grain size. When the curd has reached the desired consistency, the whey was drained off, and the curd was transferred to cylindrical moulds (15 cm high by 21 cm in diameter), which were lined with cheesecloth so as to facilitate complete draining off of whey. The curds were pressed at 3.5–4 kg/cm^2 for 4–5 h and salted in brine (19 °Baumé, temperature 8–10 °C and pH 5.3) for 16 h. Finally, the cheeses were taken to ripening chambers where they remained at a temperature of 10 °C and at 80–85% relative humidity for 210 days. Microbiological changes during manufacture and ripening of the cheeses was studied by Tornadijo et al. (2012).

Samples were taken from each of the batches after 10, 30, 60, 100 and 210 days of ripening. Each sample was made by a whole cheese of 2.5 kg, which adds up to a total of 40 cheeses analysed and transferred to the laboratory under refrigeration (4 °C). The samples were packed and stored in a freezer (−30 °C) until analysis.

2.2. Biogenic amine determination

Determination of biogenic amines was carried out following the method described by Moret et al. (2005).

The chromatographic system consisted of an HPLC Waters Alliance (Milford, Massachusetts, U.S.A.), equipped with a Waters 2695 separation module connected to a Waters 2996 photodiode array detector. The separation of biogenic amines was carried out using a Waters Atlantis Dc18 column (5 μm particle size, 150 mm × 4.6 mm I.D.) equipped with a Waters Atlantis Dc18 guard-column (5 μm particle size, 20 mm × 4.6 mm I.D.). The volume injected was 20 μl. Biogenic amines were separated by using a linear elution gradient with mobile phases A (ammonium acetate 0.1 M) and B (acetonitrile, HPLC quality). The temperature of the column was set at 40 °C ± 5 °C. Peaks were detected at 254 nm and the computer package used was Empower Version 2 by Waters™.

2.3. Statistical analysis

Statistical treatment of the results involved a one-way analysis of variance (ANOVA), with the aim of determining the effects of the ripening time and the autochthonous starter culture used to make the ewe's cheese upon the dependent variable (biogenic amines). A least squares difference (LSD) test was employed, with a confidence interval of 95% ($p < 0.05$) for which the computer package Statistica Version 6.0 (Statsoft, Tulsa, Oklahoma, U.S.A.) was used.

Thereafter, the chromatographic data obtained were analysed by multi-variant statistical techniques (Principal Component Analysis and Euclidean distance squared) by means of the statistical package Minitab 16 (Minitab Incorporated, 2010).

3. Results and discussion

The evolution of total biogenic amine content during ripening in the batches of ewe's cheese made with different starter cultures are shown in Fig. 1. In this figure, ellipses have been used to highlight significant differences ($p < 0.05$) between the batches studied.

The changes in the average total content of biogenic amines in the ewe's milk cheese (five averages corresponding to eight cheeses by five sampling points, which gives a total of 40 cheeses) increased significantly ($p < 0.001$) throughout ripening time, from 115.70 mg/kg up to 819.12 mg/kg at the end of ripening. These values were similar to those described for Idiazábal cheese (Ordoñez et al., 1997) and for Pecorino cheese (Schirone et al., 2013). However, they were lower than those recorded for other varieties, like Pecorino Abruzzese (Martuscelli et al., 2005). The low biogenic amine content found in the ewe's cheese, as compared to other cheeses of similar characteristics, is a clear indicator of good practice in hygiene during the processing and handling of the cheese.

At the end of the ripening time, phenylethylamine, spermine and tryptamine resulted to be the biogenic amines in the highest concentration in all the batches examined, which represented 81% of the total biogenic amines analysed. These results agree with those described for Pecorino Abruzzese cheese (Martuscelli et al., 2005) or Terrincho cheese (Pintado et al., 2008), in which phenylethylamine is one of the main amines. However, they differ from those established by Loizzo et al. (2013), who stated that the majority biogenic amines in ripened cheeses were tyramine, putrescine, cadaverine and histamine. Thus, the concentration of each of the biogenic amines at the end of ripening would depend on the variety of cheese involved and the multiple factors that affect the formation and accumulation of these amines.

Currently, there is no consensus as to what should be the maximum permitted concentration of biogenic amines, whether jointly or individually, in foodstuffs. Most studies have