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Variability of biogenic amine and free amino acid concentrations in regionally produced goat milk cheeses

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ABSTRACT

The free amino acid composition and biogenic amine content were analysed in pasteurised goat milk cheeses produced in different regions in Spain. These goat cheeses are made with pasteurised milk to which a mesophilic starter culture is added; they are enzymatically coagulated, uncooked, pressed cheeses. They have a firm texture with a slight but typical goat milk aroma and flavour. The total free amino acids varied markedly among the samples, ranging from 1400 to 28,000 mg kg^{-1} DM (dry matter). Of the 20 amino acids analysed, the most abundant were leucine, proline, valine, glutamic acid, lysine, glutamine, ornithine and γ-aminobutyric acid, which accounted for over 60% of the total free amino acids. The goat milk cheeses presented low concentrations of biogenic amines, the most abundant being tyramine and/or histamine, with values ranging from 4.2 to 50.7 and from 10.2 to 60.5 mg kg^{-1} DM, respectively. Total biogenic amine content ranged between 26.4 mg kg^{-1} DM and 175.1 mg kg^{-1} DM, and was always below the level that is considered dangerous for humans. Therefore, taking into consideration the concentrations of BAs, these goat milk cheeses, produced under good hygienic conditions, can be considered safe for consumers.

1. Introduction

Goat cheese consumption has increased in recent years. Goat cheese sales have grown at one of the fastest rates in the specialty food product market, and increased demand for use of these cheeses in culinary applications can been observed (Raynal-Ljutovac et al., 2011). Goat milk cheeses contain a large proportion of nutritionally valuable milk constituents, and apart from this nutritional aspect, cheeses made from goat milk are appreciated for their particular organoleptic characteristics. Moreover, goat milk may be considered an alternative milk source for people with cow milk intolerance (Tziboula-Clarke, 2003). Around thirty varieties of goat milk cheeses are manufactured in Spain; however, only six of them are protected by a designation of origin (PDO, "protected designation of origin"). This is perhaps because they have been overshadowed by the more well known, highly popular and widely-produced Manchego cheese (made with ewe milk). Many goat cheeses are currently being made with the milk of the goats from local herds in different regions of Spain, using a technology similar to that employed in the manufacture of Manchego cheese (Poveda et al., 2008). Consequently, these cheeses do not have a proper name, nor has their cheese-making technology been accurately established.

Proteolysis is the main biochemical event during cheese ripening and contributes to the development of the flavour and texture of the cheese (Upadhyay et al., 2004). First, the caseins are hydrolysed to a range of intermediate-sized peptides, which are then hydrolysed to shorter peptides and amino acids. This leads to the accumulation of free amino acids, some of which are the precursors of biogenic amines (BAs). BAs are organic, basic, low molecular-weight nitrogenous compounds, formed mainly by the decarboxylation of amino acids, which possess biological activity. BAs are harmful compounds with neuro- and vaso-active functions and should therefore be carefully monitored in food products. Their presence in foods can cause several problems for susceptible consumers, with symptoms such as headaches, vertigo, nausea and vomiting, and increased arterial blood pressure (Ladero et al., 2010). Cheese is one of the fermented foods most commonly associated with BA contamination (EFSA, 2011). The most important BAs found in goat cheese are histamine, tyramine,

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putrescine, cadaverine and 2-phenylethylamine, which are products of the decarboxylation of histidine, tyrosine, ornithine, lysine and phenylalanine, respectively. Putrescine can also be formed through deamination of agmatine (Loizzo et al., 2013). BAs can appear in cheese mainly by microbial contamination or due to the high bacterial content in raw milk cheeses. In pasteurised milk cheese, BA production has been related to the non-starter lactic acid bacteria (NSLAB) (Buňková et al., 2010), since some of this microbiota are active BA producers and are resistant to thermal treatment (Ladero et al., 2011).

The determination of BAs has been extensively reported in literature for various cheeses made with the milk of cows or ewes (Buňková et al., 2013; Flasarová et al., 2016; Piras et al., 2013; Poveda et al., 2015; Redruello et al., 2013; Renes et al., 2014; Schirone et al., 2011, 2013; Spizzirri et al., 2013). In literature that has been reviewed there are fewer studies focusing on the biogenic amine content in goat milk cheeses, (Galgano et al., 2001; Guarcello et al., 2015; Novella-Rodríguez et al., 2002, 2003, 2004; Spizzirri et al., 2013). On the other hand, there have been some studies on the characterization of regionally produced Spanish goat cheeses without PDO, in relation to the chemical composition, free fatty acid composition, microbiology and volatile compounds (Poveda and Cabezas, 2006; Poveda et al., 2008; Sánchez et al., 2005), but the biogenic amine content of these cheeses has not yet been analysed.

The aim of this work was to analyse the free amino acid composition and the biogenic amine content of goat milk cheeses manufactured in different regions of Spain and purchased in local retail stores.

2. Materials and methods

2.1. Reagents

Super-gradient HPLC grade acetonitrile and methanol were obtained from Labscan (Dublin, Ireland), and ultrapure water generated by the Milli-Q system Millipore (Bedford, MA, USA) was used. L-Cysteine, L-leucine, L-phenylalanine, L-lysine, ammonium chloride, L-histidine, cadaverine, L-arginine, histamine, L-proline, L-α-alanine, glycine, β-alanine, L-aspartic acid, L-glutamic acid, L-tyrosine, L-valine and L-serine were from Fluka Chemie (Buchs, Switzerland); diethylethoxymethylenemalonate (DEEMM), putrescine, L-glutamine, tyramine, 2-phenylethylamine and tryptamine were from Aldrich Chemie (Steinhein, Germany); L-2-aminoadipic acid, L-ornithine monohydrochloride, L-asparagine, L-threonine, γ-aminobutyric acid (GABA), L-isoleucine, L-methionine and sodium azide were from Sigma Chemie (Steinhein, Germany). Solutions of amino acids and biogenic amines were prepared with HCl 0.1 N.

2.2. Cheese samples

Eight cheeses, manufactured with pasteurised goat milk, were purchased from several local retail stores in Ciudad Real, Castilla-La Mancha, Spain (see Table 1 for sampling origin coordinates). For each brand three samples were purchased. The cheeses were manufactured with pasteurised goat milk from goats of unspecified races, following similar traditional technological procedures, which include enzyme coagulation, the use of a mesophilic commercial mixed-strain starter culture, cutting, pressing and salting. They presented a firm texture and slight but typical goat milk aroma and flavour. The precise ripening times of the cheeses were not provided by the producers. The cheeses were brought to the laboratory by refrigerated transport and, once there, were placed in a cool room at 4 °C. For each sample, a portion of approximately 250 g was taken and cut into pieces, discarding 1.5 cm from the rind, and homogenized. The analyses of pH and dry matter (DM) were made immediately. The rest of the sample was frozen at −20 °C until required for analysis. The results presented are the mean of three independent determinations carried out on three different pieces of each cheese brand (n = 3).

2.3. Physico-chemical analysis

The pH was determined by direct reading using an Ingold insertion electrode (Crison Instruments, Barcelona, Spain). DM was determined by drying to constant weight at 102 °C (IDF-FIL, 1982). Total nitrogen (TN) was analysed by the AOAC method (AOAC, 1980).

2.4. Analysis of amino acids and biogenic amines by RP-HPLC

2.4.1. Cheese sample preparation

The fraction with a molecular weight (MW) lower than 3000 Da was extracted as previously described by Poveda et al. (2015) using Centriprep Centrifugal Filter Units YM-3 with a MW cut-off of 3000 Da from Millipore (Merck KGaA, Darmstadt, Germany), following the manufacturer's instructions. For this purpose, 750 μL of internal standard (L-2-aminoadipic acid, 1 mg mL^{-1} in 0.1 N HCl) from Sigma-Aldrich (Madrid, Spain) were added to 10 mL of the pH 4.6-water soluble nitrogen (WSN) obtained as described in Poveda et al. (2003). The mixture was placed into the Centriprep unit and centrifuged for 1 h at 3000*g* at 8 °C. An aliquot of 400 μL of the extract was used for the derivatization reaction.

2.4.2. Derivatization reaction

Diethyl ethoxymethylenemalonate (DEEMM) derivatization was adapted from Gómez-Alonso et al. (2007) as follows. A mixture of 400 μL of the extract, 700 μL of 1 mol L^{-1} borate buffer

Table 1
Geographical origin and physico-chemical composition (mean ± standard deviation) of pasteurised goat milk cheeses.

Sample	Provenance (lat./long./alt. m.a.s.l.)	DM (%)	pH	TN (% DM)
C1	38°59′44″ N/1°51′21″ O/686	$58.8^{d} \pm 0.4$	$5.31^{d} \pm 0.01$	$5.38^{a,b} \pm 0.02$
C2	39°28′35″ N/6°22′20″ O/441	$59.8^{e} \pm 0.1$	$4.84^{b} \pm 0.01$	$4.83^{a} \pm 0.16$
C3	37°22′58″ N/5°58′23″ O/16	$52.8^{b} \pm 0.2$	$5.63^{f} \pm 0.01$	$6.28^{b,c} \pm 0.10$
C4	39°23′24″N/3°12′37″O/644	$62.6^{g} \pm 0.1$	$5.55^{e} \pm 0.01$	$5.76^{a,b} \pm 0.19$
C5	41°23′19″ N/2°09′32″ E/47	$61.5^{f} \pm 0.3$	$5.36^{d} \pm 0.01$	$5.59^{a,b} \pm 0.20$
C6	41°30′22″ N/5°44′40″ O/662	$65.4^{h} \pm 0.3$	$5.21^{c} \pm 0.02$	$5.64^{a,b} \pm 0.26$
C7	38°59′10″ N/3°55′44″ O/633	$52.2^{a} \pm 0.5$	$4.77^{a} \pm 0.06$	$6.76^{c} \pm 0.12$
C8	39°02′59″N/4°03′37″ O/603	$57.8^{c} \pm 0.0$	$5.18^{c} \pm 0.01$	$5.58^{a,b} \pm 0.16$

Results presented are the mean of three independent determinations carried out in three different samples of each cheese brand (n = 3).
DM: dry matter; TN: total nitrogen.
[a–h]Different superscripts in the same column indicate significant statistical differences ($P<0.05$) according to the Student-Newman-Keuls test.