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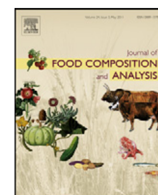

Original Research Article

Determination of biogenic amines in different cheese samples by LC with evaporative light scattering detector

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ABSTRACT

The paper presents the application of liquid chromatography coupled with evaporative light scattering detector (LC-ELSD) for the determination of biogenic amines in different cheese samples, as their presence and relative amounts give useful information about freshness, level of ripening and quality of storage. Forty samples from different types of milk – hard-ripened, ripened and unripened – were considered. Results showed that the amine contents varied in relation to the manufacturing process, the highest concentration being in hard-ripened cheeses followed by ripened and then unripened. In hard-ripened cheeses amines were β-phenylethylamine (PHE) (69.8–136.6 mg kg^{-1}), tyramine (TYR) (19.7–147.1 mg kg^{-1}), spermidine (SPD) (nd–73.1 mg kg^{-1}), cadaverine (CAD) (nd–64.7 mg kg^{-1}), histamine (HIS) (17.6–48.2 mg kg^{-1}), spermine (SPM) (nd–47.4 mg kg^{-1}), putrescine (PUT) (nd–44.1 mg kg^{-1}) and agmatine (AGM) (nd–4.2 mg kg^{-1}); while in ripened cheese TYR (nd–116.7 mg kg^{-1}), PUT (nd–82.9 mg kg^{-1}), HIS (nd–57.7 mg kg^{-1}), PHE (nd–51.1 mg kg^{-1}), SPD (nd–31.5 mg kg^{-1}), CAD (nd–30.7 mg kg^{-1}), SPM (nd–26.9 mg kg^{-1}) and AGM (nd–4.8 mg kg^{-1}). On the basis of literature limits, in this study only hard ripened cheeses could represent a possible risk for consumers as they exceeded a proposed limit for PHE and total biogenic amines amount.

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1. Introduction

Biogenic amines (BAs) are basic nitrogenous compounds formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones (Silla-Santos, 1996; Ten Brink et al., 1990). The decarboxylation process can proceed through two biochemical pathways which are activity of endogenous decarboxylase enzymes naturally occurring in food or activity of exogenous enzymes released by various microorganisms. So, in virtually all foods that contains proteins or free amino acids and subjected to conditions enabling microbial or biochemical activity BAs formation can be expected. However, the total amount of the different amines is strongly variable depending on the nature of food and the microorganisms involved (Halasz et al., 1994; Stratton et al., 1991; Ruiz-Capillas and Jimenez-Colmenero, 2004). The presence of BAs in non-fermented foods generally indicates inadequate or prolonged storage; on the other hand, their presence in fermented foods could be unavoidable due to the diffusion of decarboxylases among lactic acid bacteria.

Among foods, cheese is an ideal substrate for amine production as its manufacturing process involves the availability of free amino acids produced as an outcome of proteolysis levels, but also the possible presence of decarboxylase-positive microorganisms and the environmental conditions that allow their growth as well as the presence of suitable cofactors (pyridoxal phosphate) (Curtin and McSweeney, 2004; Bernardeau et al., 2008; Pereira et al., 2001; Pinho et al., 2004; Linares et al., 2011). Other factors affecting the production of BAs in cheeses include the presence of spoiling microorganisms and the synergistic effects between microorganisms (Silla-Santos, 1996; Marino et al., 2000).

In particular, it has been reported that increases in the amine content of cheese may be attributable to various micro-organisms possessing amino acid decarboxylase activity found among starter lactic acid bacteria, non-starter lactic acid bacteria and/or other spontaneous microflora (Halasz et al., 1994). However, it is difficult to find a straight correlation between microbial counts and BA content in cheese, because amine-producing abilities of different strains of various bacteria differ widely (Linares et al., 2011). Moreover several extrinsic processing factors may also play an important role, namely, pasteurization of milk, pH, salt-in-moisture levels and ripening temperature. In particular, the pH of cheese (5.0–6.5) is optimum for the activity of most decarboxylases and it has been found that the production of BAs

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is accelerated by high temperatures during production and manufacture of cheese and by the prolonged aging process (Gardini et al., 2001; Santos et al., 2003; Gennaro et al., 2003; Pinho et al., 2001; Bunková et al., 2010; Martuscelli et al., 2005; Marino et al., 2008; Novella-Rodrìguez et al., 2002a).

Although several BAs can play important roles in many human physiological functions (Kalac, 2009; Bardócz et al., 1995), their presence in foods is always undesirable because if absorbed at too high concentration, they may induce headaches, respiratory distress, heart palpitations, hypo- or hypertension and several allergenic disorders (Shalaby, 1996; Taylor, 1986; McCabe-Sellers et al., 2006; Jansen et al., 2003). These compounds can represent a serious health hazard for humans and animals when present in food in significant amounts, or ingested in the presence of potentiating factors, such as amine oxidase-inhibiting drugs, alcohol and gastrointestinal diseases (Stratton et al., 1991). Not all amines are equally toxic; HIS, TYR and PHE are of major concern (Shalaby, 1996). Cases of TYR intoxication have occurred subsequent to the consumption of cheese (Stratton et al., 1991; Taylor, 1985, 1986) and the term "cheese reaction" has been coined to refer to it (Silla-Santos, 1996). PUT and CAD may potentiate the toxic effects of HIS and TYR by inhibiting monoamine oxidase, diamine oxidase, and hydroxymethyl transferase (Bardócz et al., 1995; Straub et al., 1995).

As the presence of BAs has great impact on food quality and safety, during recent years different methods have been developed for their identification and quantitative determination. For the separation of BAs, various chromatographic techniques such as thin-layer chromatography, gas chromatography, liquid chromatography (LC) as well as capillary electrophoretic methods are used (Önal, 2007). Since many amines show neither natural UV absorption nor fluorescence, most LC methods require that amines should be derivatized before detection (Innocente et al., 2007; Soufleros et al., 2007; Krause et al., 1995; Özdestan and Üren, 2009; Chiacchierini et al., 2006; Busto et al., 1996; Lozanov et al., 2004; Köros et al., 2008; Pereira et al., 2008; Vandenabeele et al., 1998). LC methods with electrochemical detection have been also employed (Favaro et al., 2007) as well as ultra pressure liquid chromatography techniques (Latorre-Moratallaa et al., 2009; Dadáková et al., 2009; Mayer et al., 2010).

More recently, a new LC method with evaporative light scattering detector (ELSD) has been validated for BA determination in cheese (Restuccia et al., 2011), and the main advantage has been the elimination of the derivatization procedure drawbacks. In this regard, although LC methods coupled with mass spectrometry detection without a previous derivatization step (Gosetti et al., 2007; Gianotti et al., 2008; Saccani et al., 2005) have been developed to quantify BAs in cheese, a severe matrix effect has been reported. Moreover ELSD is more affordable than mass spectrometry, and is also compatible with a broad range of solvents and gradient elution.

The aim of this work is application of an improved LC-ELSD method for quantitative determination of HIS, SPD, SPE, TYR, PUT, CAD, AGM and PEA in 40 commercial cheese samples with different technological characteristics (i.e. kind of milk, milk pasteurization and ripening times). In comparison with the previous study (Restuccia et al., 2011) two other amines have been added and LC parameters have been deeply modified to permit suitable quantitative determination. In particular CAD and AGM were considered: the first because it's one of the most abundant BAs present in cheese and the second because its LC–UV quantitative determination is very difficult to accomplish. Moreover as the first study was applied only to one cheese, the application to many samples which are expected to contain very different BAs amounts could assess the effectiveness of the method.

2. Materials and methods

2.1. Samples

Commercial cheese samples ($n = 40$) of different types were taken from local retail. Italian cheeses with Protected Designation of Origin (PDO) were chosen, as PDO requires that cheese be produced in a defined area under a specific standard of identity. However, other cheese samples without PDO were also considered. In particular, the following cheeses were analyzed: three Parmigiano Reggiano PDO ripened for 30 months (1a–c), two Parmigiano Reggiano PDO ripened for 24 months (2a–b), three Grana Padano PDO ripened for 22 months (3a–c), two grated Parmigiano Reggiano PDO (4a–b), two Grated Grana Padano PDO (5a–b), Provolone Valpadana PDO (6), Pecorino Romano PDO (7), Pecorino Crotonese (8), three ripened goat cheese (9a–c), Emmentaler Switzerland AOC (10), Asiago PDO (11), Taleggio PDO (12), Caciocavallo Silano PDO (13), Montasio PDO (14), Fontina PDO (15), Bel Paese (16), three Caciotta (17a–c), three unripened goat cheeses (18a–c), three cheese spreads (19a–c), three Mozzarella (20a–c), Mozzarella di Bufala Campana PDO (21), Robiola di Roccaverano PDO (22), and Ricotta Romana PDO (23).

Different cheese samples were evaluated in the study. Unripened and ripened cheeses were from raw and pasteurized milk, while hard-ripened cheeses were obtained only from raw milk. Ricotta Romana is an unripened whey cheese and is produced heating at 50–60 °C the whey separated from the ewe milk during the production of Pecorino Romano cheese. Unripened cheeses were manufactured from cow, water buffalo and goat milk; ripened cheeses were obtained from cow, ewe and goat milk and hard ripened cheeses were all from cow milk. Main characteristics of cheese samples are summarized in Table 1.

For BA determination, all cheese samples were cut in half, and a slice 2–3 cm thick was separated from each half. The outer section of each slice (1–2 cm) was removed and discarded; the remaining was reduced to small pieces of about 3 mm of diameter or grated (only extra-hard cheeses); then sample were mixed and homogenized thoroughly into pools. 5 g of the homogenized sample were then subjected to the extraction procedure. Only for samples 4a–b and 5a–b (already grated) and 19, 22 and 23 (slicing or grating were not possible to achieve), 5 g of cheese where directly subjected to extraction procedure.

As far as BAs extraction from cheese samples and SPE purification of the extracts is concerned, the applied protocol have already reported (Restuccia et al., 2011). Briefly, 20 mL of hydrochloric acid 0.1 M were added to about 5.0 g of cheese (or cheese spiked with standard solution), in a 50.0 mL test tube. The mixture was homogenized (vortex at 40 Hz for 40 min), centrifuged (12,000 × g for 25 min), filtered (syringe filter 0.20 μm), collected in a plastic vial and purified by SPE on a C_{18} sorbent (Loading: 4.0 mL of the sample; washing: 2.0 mL of water; Eluting: 2.0 mL (two times) of CH_3OH). The eluting solution, dried up with nitrogen gas and the residue re-dissolved in a plastic test tube with 800 μL of ultrapure water for LC-ELSD analysis. Recovery experiments were performed spiking, before the extraction procedure, samples 2a, 6 and 18b with different aliquots of a BAs standard mix in order to evaluate the method performances at three different levels of concentration. BAs amount added to cheeses were of the same order of magnitude of the supposed BAs concentration of each sample. In particular, sample 2a, sample 6 and sample 18b were spiked, respectively, with 1.0 mL, 0.6 mL and 0.15 mL of a standard solution mixture at concentration of 350 mg L^{-1}.

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