International Dairy Journal 61 (2016) 189–195

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Effect of milk pasteurisation and of ripening in a cave on biogenic amine content and sensory properties of a pecorino cheese

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ARTICLE INFO

Article history:
Received 8 October 2015
Received in revised form
31 May 2016
Accepted 31 May 2016
Available online 16 June 2016

ABSTRACT

Ewes' milk cheeses were evaluated with respect to the influence of pasteurisation and of ripening in a cave on the biogenic amine (BA) content and sensory properties. Both factors significantly influenced the BA content, with higher BA concentrations (on average >1500 mg kg^{-1} total; 850 mg kg^{-1} of tyramine) in cheeses manufactured with raw milk and partly ripened in a cave. Milk pasteurisation effectively limited BA formation both qualitatively and quantitatively, but still allowed the accumulation of notable BA levels in cave ripened cheeses. Thus, milk pasteurisation 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, which 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 pasteurisation 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 pasteurisation 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 pasteurised milk and a starter culture. However, in experimental

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http://dx.doi.org/10.1016/j.idairyj.2016.05.007
0958-6946/

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, Veciana-Nogués, Trujillo-Mesa, & Vidal-Carou, 2002; Novella-Rodríguez et al., 2003; 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 with 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 in which 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 pasteurisation 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 ewes' 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 1500 L of milk, one with pasteurised (70 °C, 40 s) milk 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 pasteurisation and ripening conditions.

2.2. Cheese samples

Two samples of curd were collected for each cheesemaking trial, one manufactured with pasteurised milk and one with raw milk. For each cheesemaking trial, 6 cheese samples were collected after 2 months of ripening: 3 made with pasteurised 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 pasteurised 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. Biogenic amine 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 high performance liquid chromatography (HPLC) analysis, using 1,7-diaminoheptane as an internal standard, dansyl-chloride for pre-column derivatisation, a RP Gemini C18 column (250 mm × 4.60 mm, 5 μm) (Phenomenex, Torrance, CA, USA) and a Jasco HPLC apparatus (Jasco Corporation, Tokyo, Japan). BA extraction, derivatisation, and HPLC analyses were performed following the procedure described by Innocente, Biasutti, Padovese, and 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 homogenised 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 (VRBGA; 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 analysed 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: pasteurisation 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 panellists were involved in 6 sessions. For the quantitative descriptive analysis (QDA) a panel was formed with 8 trained panellists (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 (ISO, 2012). Four training sessions were carried out on the quantification of sensory attributes in cheese. In 5 subsequent sessions, each panellist 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: ewes' milk, bitter, sweet, piquant, animal/stable, nutty, butter, and "acidity feel" (defined as a fermented lactic-acid aftertaste). The 4 texture characteristics were: hardness, granularity, fracturability, and fatness.