Food Chemistry 112 (2009) 1053–1059

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Volatile fraction of DOP "Castelo Branco" cheese: Influence of breed

Isabel M.P.L.V.O. Ferreira [a,*], Olívia Pinho [a,b], Paulo Sampaio [a]

[a] REQUIMTE – Serviço de Bromatologia, Faculdade de Farmácia da Universidade do Porto, Rua Anibal Cunha 164, 4099-030 Porto, Portugal
[b] Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

ARTICLE INFO

Article history:
Received 7 April 2008
Received in revised form 18 June 2008
Accepted 21 June 2008

Keywords:
Cheese
Ovine race
Headspace analysis
Solid-phase microextraction
Volatile profile
Flavour

ABSTRACT

"Castelo Branco" cheese is a Portuguese DOP cheese made from raw ewe's milk coagulated with *Cynara cardunculus*, ripened for at least 40 days. "Merino da Beira Baixa" pure race is frequently used to produce milk for this cheese, however, exotic races such as Assaf and crusade of these two races are also used. The aim of this work has been to compare the volatile profile and sensory characteristics of DOP "Castelo Branco" cheeses manufactured during winter season with milk of breeds from Merino, Assaf and crusade of these two races and identify volatile compounds that can distinguish these cheeses.
Volatile compounds profile was assayed by SPME–GC–MS. A total of 67 volatile compounds were separated and identified. The volatile profiles of the three types of cheeses differed significantly. Descriptive analysis and triangle tests confirmed that these cheeses presented significantly different sensory characteristics. Discriminant analysis showed that specific volatile components seemed to distinguish specific cheeses.

1. Introduction

"Castelo Branco" cheese is a traditional product of the interior of Portugal. In 1994 it was labelled with PDO (protected denomination of origin) DR II (1994). Production of "Castelo Branco" cheese remains faithful to tradition. It is made from whole raw ewe's milk. "Merino da Beira Baixa" pure race is frequently used to produce milk for this cheese, however, exotic races such as Assaf and crusade of these two races are also used. After coagulation with vegetable coagulant at 30 °C (15–25 min), the curd is slightly drained and placed in moulds for pressing and salting according to the traditional method (Freitas & Malcata, 2000).

Cheese volatile fraction and consequently sensory characteristics are affected by climatic conditions and raw milk quality, which depends on the animal species, raw, breed, feed and farming. The adventitious microflora of the raw milk will also play a relevant role (Collomb, Butikofer, Spahni, Jeangreos, & Bosset, 1999; Fernàndez-Garcia, Carbonell, Calzada, & Nunez, 2006; Freitas & Malcata, 2000; Nàjera, Barròn, & Barcina, 1993; Perea et al., 2000; Tavaria, Dahl, Carballo, & Malcata, 2002; Tavaria, Ferreira, & Malcata, 2004).

Cheese lipolysis by microorganisms and native milk lipases is an important phenomenon in the development of flavour during cheese ripening. This is especially important in raw milk cheese, where the native lipase is not deactivated by pasteurisation. The major flavour of these cheeses comes from short and medium-chain FFA (Nàjera et al., 1993). The strong odours of these compounds contribute to the cheese lipolysed aroma (Chávarri et al., 1999; Fox & Wallace, 1997; House & Acree, 2002; Kalantopoulos, 1993; Macedo & Malcata, 1996; Sousa & Malcata, 1997; Tavaria et al., 2004). In addition, they serve as precursors of other impactful flavour compounds such as esters and methyl ketones (Urbach, 1993). Odour descriptors associated with typical aroma of "Castelo Branco cheese" are "acidic", "sheepy-like" and "pungent" notes.

To characterise the volatile compounds of a cheese at a given time, gas chromatography coupled to mass spectrometry is the method of choice. The isolation of compounds from cheese matrix by headspace solid phase microextraction (HS–SPME) has been found preferable to other techniques because it is solvent free, and presents high sensitivity and limited risk of artifacts (Kataoka, Lord, & Pawliszyn, 2000; Pinho, Ferreira, & Ferreira, 2002, 2004; Pinho, Pérès, & Ferreira, 2003; Tavaria et al., 2004).

The choice of ovine race can influence milk composition. Consequently, differences would be expected in the volatile pattern and flavour of "Castelo Branco" cheeses manufactured with milk from "Merino de Beira Baixa" pure race, Assaf exotic race and crusade of these two races. However, no studies were found with respect to the volatile fraction of this cheese and the influence of ovine race in the sensory characteristics of this cheese (Freitas & Malcata, 2000).

The aim of this work has been to compare the volatile profile and sensory characteristics of DOP "Castelo Branco" cheeses manufactured during winter season with milk of breeds from Merino, Assaf and crusade of these two races and identify volatile compounds that can distinguish these cheeses. In addition, other analyses,

* Corresponding author.
E-mail address: isabel.ferreira@ff.up.pt (I.M.P.L.V.O. Ferreira).

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doi:10.1016/j.foodchem.2008.06.048

involving the evaluation of major components and microbiological safety, were carried out in order to obtain a more complete characterisation of this product.

2. Materials and methods

2.1. Sampling

Three lots of "Castelo Branco" cheeses were manufactured, during the winter season, in a certified dairy plant according to the specifications of its Denomination of Origin Regulatory Board (DR II, 1994) each lot included one batch of cheeses with milk from Merino da Beira Baixa breed, one batch of cheeses with milk from Assaf breed, and one batch of cheeses with milk from Crusade of the two races breed, respectively. These cheeses are named throughout this work as MCB (Merino Castelo Branco cheese), ACB (Assaf Castelo Branco cheese) and CCB (Crusade Castelo Branco cheese).

Breeds from Merino da Beira Baixa, Assaf, and Crusade of these two races were grown under controlled identical conditions, representative of the ovine production in the region. Lactating ewes were in similar physiological conditions. A similar diet was given to the three breeds before milk production and included pasture and the same brand of concentrate.

Aliquots of milk of each breed were taken before cheese manufacture for physicochemical and microorganism analyses.

For each cheese preparation 25 L of milk was coagulated with artisan-produced vegetable rennet (mixing flowers with water at a ratio of 5 g per 95 ml allowed to stand 1 h) added to milk at a ratio of 5 ml per 25 L of milk. The resulting curds were cut, slightly drained and placed in moulds, where they were pressed to help in expression of the remaining whey. The cheeses were salted upon unmoulding via leaving for 20 h in a saturated solution of NaCl, and placed (without any sort of packaging, or wropping for that matter) in ripening chambers held at 10–12 °C and 88–89% relative humidity. All cheeses were ripened for 64 days in the certified dairy plant under similar conditions; the minimum recommended ripening time is 40 days.

2.2. Microorganism isolation and enumeration

Microbiological analyses of milk and cheese samples were performed to guaranty the safety of the products. Milk of the three breeds was analysed for enumeration of mesophilic microflora (ISO 4833, 2003), *Staphylococcus aureus* (NP 4400-1, 2002), *Escherichia coli* (NF ISO 16649-2, 2001) and *Listeria monocytogenes* detection (ISO 11290-1, 1996).

Microbiological analysis of cheese samples manufactured with milk from the three breeds were carried at the end of ripening for enumeration of Enterobacteriaceae (ISO 21528-2, 2004), *Pseudomonas* spp. (NF V 04-504, 1998), *Staphylococcus* coagulase positive (NP 4400-1, 2002), *E. coli* (NF ISO 16649-2, 2001), mesophilic microflora lactic (NF ISO 15214, 1998) and aerobic (ISO 4833, 2003) and *L. monocytogenes* detection (ISO 11290-1, 1996). Lactococci and lactobacilli were grown anaerobically (Gas-Pak anaerobic system, from BBL, Cockeysville, MD, USA) on M17 Agar (Lab M, Bury, UK) and Rogosa Agar (Lab M), at 30 °C for 2 and 5 d, respectively.

2.3. Physicochemical analysis

Major nutrients of ovine milk from the three breeds were determined. Fat content was quantified according to ISO method (ISO 2446, 1976). Casein content was evaluated by HPLC/UV (Ferreira, Mendes, & Ferreira, 2001) and lactose content was determined by HPLC/light scattering (Nogueira, Silva, Ferreira, & Trugo, 2005).

2.4. Extraction and analyses of cheese volatile compounds

2.4.1. Sample preparation

Each cheese was divided in four peaces and an aliquot of each one was analyzed. The cheese samples were finely grated; prior to grating, a layer of 0.3 cm was removed from the cheese surface in order to minimise the sampling of those volatile compounds that might have eventually migrated from the environment. For each cheese, a 3 g sample taken was and placed in a 15 ml vial subsequently sealed with PTFE-silicone septa (Supelco, Bellefonte, PA, USA).

2.4.2. Headspace and SPME

Carboxen/PDMS fibre (Supelco, Bellefonte, PA) was used for extraction of cheese volatile compounds. The conditions used for characterisation of "Castelo Branco" ewe cheese were: sample vial equilibration at 20 °C for 20 min, followed by CAR/PDMS fibre exposure to the headspace above the sample for 30 min and finally thermal desorption of the adsorbed substances in the injector port GC–MS analysis (Pinho et al., 2004).

The analyses were performed using a Hewlett–Packard (HP), model 6890, GC fitted with a splitless injector suitable for SPME analysis and Agilent 5973 mass spectrometer (MS) detector. Helium was used as the carrier gas with a flow rate of 1 ml/min. The components were separated on a SPB-5 capillary column 60 m × 0.32 mm × 1.0 μm-film thickness, (Supelco, Bellefonte, PA). The oven temperature program was 5 min at 40 °C and then 3 °C min^{-1} to 200 °C for 5 min. The injector temperature was 290 °C. Detection was by mass spectrometry on the total ion current obtained by electron impact at 70 eV. When possible, the identity of the volatile compound was confirmed using an authentic standard. The constituents were also identified by comparing the experimental spectra with spectra from Nist' 98 data bank (NIST/EPA/NISH Massa Spectral Library, version 1.6, United States). Based on the peak resolution, their areas were either calculated from the total ion current or estimated from the integrations performed on selected ions. The resulting peak areas were expressed in arbitrary units of area. Propionic, butyric, isovaleric, isobutyric, caproic, caprylic, capric, and lauric acids, as well as ethyl butanoate, hexanoate, octanoate, decanoate, and dodecanoate (purity: 99%) were purchased from Sigma Chemical Co. (St. Louis, MO); acetic acid, propanone, butan-2-one, ethanol, propan-2-ol and benzoic acid were from Merck (Darmstadt, Germany).

2.5. Cheese sensory analysis

Triangle tests to evaluate if a difference exists between sensory characteristics of MCB, ACB and CCB cheeses were performed with sixty-three non trained assessors according to ISO 4120 (2004). Cheese samples were coded in a uniform manner, using three-digit numbers, chosen at random. Each trial was composed of three samples, each with a different code. Two of the samples were the same cheese and one was a different cheese. All the possible sequences of the three products were given to the assessors. Each assessor indicated which one of the three samples was different from the other two. All assessors evaluated all the products. Statistical analysis of results were performed for an $\alpha = 0.05$.

Additionally, a panel composed of seven members performed quantitative descriptive analysis. Subjects were selected (two sessions) for their sensory ability and trained for descriptive analysis according to the guidelines in the ISO 6564 (1985) standard flavour profiles. As suggested by Carbonell, Nuñes, & Fernández-Garcia (2002), panellists were asked to give a classification on 0- to 7-point scale for quality and intensity of odour and aroma, fatty aspect, sheep-like odour, acid, pungent and rancid notes.