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Analytical Methods

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

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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,

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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.

2.6. Statistical analyses

Data are presented as the mean ± standard deviation. The results were statistically analysed by analysis of variance (ANOVA). Differences were considered significant for $p < 0.05$. Statistical analyses were all performed with SPSS for Windows version 14 (SPSS Inc., Chicago, IL). Exploration of data and discriminant analysis by stepwise method were performed with SPSS for Windows version 13 (SPSS, Chicago, IL, USA).

3. Results and discussion

3.1. Microorganism isolation and enumeration of milks and cheeses

Microbiological control of milk and cheese samples was performed to guarantee safety of the final product that would be used by panellists. No significant differences were observed concerning the numbers of mesophilic aerobic microorganisms (ranged between 5.342 and 6.079 log cfu ml^{-1}), *S. aureus* (<1 log cfu ml^{-1}), and *E. coli* (ranged between 1 and 1.839 log cfu ml^{-1}) obtained for ovine milk samples from Merino, Assaf and Crusade breeds.

Enterobacteriaceae, *Pseudomonas* spp., Mesophilic lactic acid bacteria, Mesophilic aerobic microorganisms, *S. aureus* and, *E.* coli evaluated in "Castelo Branco" cheeses at 64 days of ripening evaluated. In general, the test of significance revealed no significant differences in microbiological composition of milk from the three breeds and respective ripened cheeses. As expected no *L. monocytogenes* were found on milk neither on cheese. The results obtained indicated that cheeses were safe for use in sensory analysis.

3.2. Ovine milk physicochemical characteristics

Quality control of milk used for cheese preparation was performed by the analysis of fat, casein and lactose contents from Merino milk, Assaf milk and milk from crusade of these two races. Significant differences were found between fat content of milk from three breeds. Milk from "Merino da Beira Baixa" breed presented higher fat content (7.15 ± 0.05 g/100 ml) and milk from Assaf breed presented lower fat content (5.05 ± 0.10 g/100 ml). No significant differences were found for lactose contents in milk from the three breeds (mean value 4.28 ± 0.23 g/100 ml) and for casein contents in milk from the three breeds (mean value 6.05 ± 0.24 g/100 ml). As observed the main difference in the composition of the milk from the three breeds is its fat content.

3.3. Cheese volatile profile

The HE–SPME extraction and the chromatographic method allowed the determination of 67 volatile compounds in the headspace of "Castelo Branco" cheeses MCB, ACB, CCB. A total of 11 fatty acids, 9 ketones, 1 aldehyde, 10 alcohols, 12 esters, 7 hydrocarbons, 8 aromatic compounds, 6 sulphur compounds, 2 alicyclic compounds and 1 ether were identified. The analytical repeatability was fully satisfactory no value of CV over 5% was found.

The volatile compounds identified were grouped by chemical class, to simplify a comparison of the different samples. The gas chromatographic profiles of "Castelo Branco" cheese volatiles expressed in percentage of TIC area of each chemical family showed significant differences between MCB, ACB, CCB cheeses concerning acids, carbonyl compounds, alcohols and esters. MCB cheeses presented higher content of acids, carbonyl compounds and esters. Hydrocarbons, aromatic sulphur, alicyclic and ether compounds were present in low amounts. ACB cheeses presented higher content of alcohols and CCB cheeses presented an intermediate content (Fig. 1).

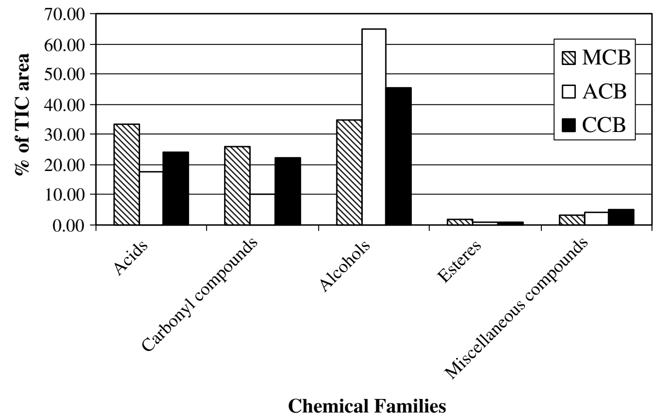

Fig. 1. Distribution of volatiles in MCB, ACB and CCB cheeses by chemical families. Miscellaneous compounds include: hydrocarbon, aromatic, sulphur, alicyclic and ether compounds.

3.3.1. Acid compounds

Acid compounds identified by SPME–GC–MS analysis of the volatile fraction of "Castelo Branco" cheese according to ovine milk origin and relative areas achieved are presented in Table 1. It is described that the main source of free fatty acids is lypolysis, but they can also result of the metabolism of lactose, be biosynthesised directly from acetyl–CoA, or formed from amino acid conversion (for example, valine and leucine) (Tavaria et al., 2004). The higher content of free fatty acids on MCB cheeses suggests that they result mainly from fat lipolysis. Free fatty acids have an important contribution to cheese aroma, either directly by their aromatic notes, or as precursors of carbonyl compounds, alcohols, alkanes and esters. Thus, free fatty acids can be involved in cheese aroma or in a rancidity defect when they are present in very large amounts (Chávarri et al., 1999; Fox & Wallace, 1997; House & Acree, 2002; Kalantopoulos, 1993; Macedo & Malcata, 1996; Sousa & Malcata, 1997; Tavaria et al., 2004). Each compound has a characteristic odourous note. Eleven acids were identified in MCB, ACB and CCB cheeses; eight were simultaneously present in the three groups of cheeses including acetic, propanoic, 2-methyl-propanoic, butanoic, pentanoic, 2-methylbutanoic, hexanoic and octanoic acids. It should be pinpointed that MCB presented all fatty acids and in general higher relative areas were achieved comparing with ACB and CCB cheeses. These compounds are related with the sensory characteristics of typical aroma of "Castelo Branco" cheese, sheepy-like odour. Similar relative areas were observed for acetic, pentanoic and hex-3-enoic acids in the three types of cheese.

3.3.2. Carbonyl compounds

Ketones are intermediate compounds which may be reduced to secondary alcohols. Methyl ketones are produced from fatty acids by oxidative degradation. The production of methyl ketones involves oxidation of fatty acids to β-ketoacids, which are then decarboxylated to corresponding methyl ketones with one carbon atom less, mainly from $C_{6:0}$ to $C_{12:0}$ fatty acids. Due to their typical odours, methyl ketones have a key role in the flavour of ripened cheese as result of their much lower perception thresholds. Nine methyl ketones were identified (Table 1). Only butan-2-one and 3-hydroxybutan-2-one were present simultaneously in the three groups of cheeses, however, at different percentages. Enzymes from bacteria present in raw milk reduce 3-hydroxybutan-2-one to butan-2-one and butan-2-ol, compounds found in MCB, ACB and CCB cheeses, similar situation was observed in La Serena cheese, a Spanish cheese made from Merino ewe raw milk coagulated with *Cynara cardunculus* (Carbonell et al., 2002) and in other raw milk cheeses (Izco & Torre, 2000). ACB cheeses presented poor

Table 1
Acids, ketones, aldehydes, alcohols and esters identified by SPME–GC–MS analysis of the volatile fraction of "Castelo Branco" cheese according to ovine milk

Compound	Flavour note[A]	t_R (min)[B]	TIC/ion[C]	Relative areas achieved with different cheeses		
				MCB	ACB	CCB
Acids						
Acetic acid	Vinegar, pungent	9.423	60	85	100	94
Propanoic acid	Vinegar, pungent	13.54	74	32[a,b]	24[a]	100
2-Methyl-propanoic acid	Sweet, apple-like, rancid butter	17.983	73	100[a]	76[c]	52[b]
Butanoic acid	Rancid, cheesy, putrid, sweaty	19.377	60	100[a]	33[b]	57[b]
Pentanoic acid	Cheesy-like, sweaty, rancid, waxy	22.766	60	63[a]	100[a]	98[a]
3-Methylbutanoic acid	Swiss cheese, waxy, sweaty, old socks, fecal	23.346	TIC	100[a]	0[b]	0[b]
2-Methylbutanoic acid	Fruity, sour, sweaty	23.795	74	100[a]	46[b]	54[b]
Hexanoic acid	Pungent, blue cheese, goat-like	30.179	TIC	100[a]	78[a,b]	67[b]
Hex-3-enoic acid		30.418	TIC	100[a]	0[c]	68[b]
Octanoic acid	Goaty, waxy, soapy, body odour	40.613	TIC	89[a]	100[a]	11[b]
Decanoic acid	rancid	50.131	TIC	100[a]	0[b]	0[b]
Ketones						
Propanone	Ethereal, powerful, fruity	5.891	TIC	4[a]	0[a]	100[b]
Butan-2,3-dione	Buttery	8.383	TIC	0[a]	100[c]	52[b]
3-Methylbutan-2-one	Camphor	8.411	TIC	100[a]	0[b]	0[b]
Butan-2-one	Acetone, etheric	8.760	TIC	100[a]	43[b]	68[b]
Pentan-2-one	Fruity, acetone, sweet, etheral	12.934	43	85[a]	0[b]	100[a]
3-Hydroxybutan-2-one	Buttery	14.131	43	68[a]	40[c]	100[b]
4-Methylpentan-2-one		15.972	TIC	49[a]	0[c]	100[b]
Heptane-2-one	Floral, fruity	25.260	TIC	100[a]	0[c]	89[b]
Nonan-2-one	Musty, fruity, floral	36.990	TIC	100[a]	0[c]	75[b]
Aldehydes						
3-Methylbutanal	Dark chocolate, malt, green	11.310	TIC	31[a]	100[b]	48[a]
Alcohols						
Ethanol	Alcohol, mild	5.294	TIC	83[a]	55[b]	100[a]
Propan-2-ol		5.932	TIC	0[a]	100[b]	0[a]
Propan-1-ol	Alcohol, sweet	7.446	TIC	68[a]	100[b]	88[a,b]
Butan-2-ol	Alcohol	8.993	59	26[a]	100[a]	31[a]
2-Methylpropan-1-ol		10.046	TIC	81[a]	100[a]	56[b]
Butan-1-ol	Sweet, fruity	11.751	TIC	13[a]	100[c]	29[b]
Pentan-2-ol	Mild green, fused oil	13.546	45	0[a]	100[b]	0[a]
3-Methylbutan-1-ol	Fruity, alcohol	15.637	TIC	54[a]	100[c]	68[b]
Butan-2,3-diol	Fruity	18.291	45	68[a]	17[c]	100[b]
Heptan-2-ol	Earthy oil	25.82	TIC	0[a]	100[b]	22[a]
Esters						
Ethyl ethanoate	Solvent, pineapple, fruity	9.450	TIC	100[a]	0[b]	0[b]
Ethyl propanoate		14.308	57	100[a]	76[b]	92[a,b]
Propyl acetate		14.445	43	92[a]	99[a]	100[a]
Ethyl butanoate	Pineapple, sweet, banana	19.665	60	79[a]	100[a]	87[a]
3-Methylbutyl ethanoate	Fruity, banana	24.380	TIC	65[a]	100[c]	53[a]
2-Methylpropyl 2-methylpropanoate		28.100	TIC	0[a]	100[b]	0[a]
Propyl pentanoate		31.236	TIC	100[a]	0[b]	0[b]
Ethyl hexanoate	Pineapple, apple powerful	31.679	TIC	100[a]	98[a]	99[a]
Butyl 3-methyl butanoate		34.925	TIC	0[a]	100[c]	78[b]
3-Methylbutyl butanoate		34.939	87	100[a]	0[b]	0[b]
Pentyl-3-oxobutanoate		37.697	TIC	100[a]	0[b]	0[b]
Ethyl octanoate	Apricot, win, floral	42.385	TIC	86[a]	89[a]	100[a]

[A] The sensory properties of the most characteristic compounds are indicated.
[B] Retention time.
[C] When the resolution was not perfect chromatographic peak areas were not calculated from the ion current (TIC) but were estimated from integrations performed on the specified indicated ions.
[a,b,c] Means in lines without common letters are significantly different ($p < 0.05$).

composition of carbonyl compounds when compared with MCB and CCB cheeses (Table 1). MCB and CCB cheeses presented similar profile of some ketones.

Aldehydes are unstable compounds that are reduced to alcohols or oxidised to acids, only one aldehyde, 3-methylbutanal, was identified. It was present in the three types of cheeses. However, higher abundance was found in ACB cheeses (Table 1).

3.3.3. Alcohols

The strong reducing conditions in cheese flavour the formation of alcohols from aldehydes and ketones, following reaction pathways which involve alcohols dehydrogenases. Ten alcohols were identified in MCB, ACB and CCB cheeses, but only seven, ethanol, propan-1-ol, butan-2-ol, 2-methylpropan-1-ol butan-1-ol, 3-methylbutan-1-ol, butan-2,3-diol, were simultaneously present (Table 1). Abundances of alcohols were significantly ($p < 0.01$) different between MCB, ACB and CCB cheeses. ACB cheeses presented higher abundance of alcohol content. However, alcohols have a limited role in the aroma of cheese despite its high levels, but it contributes to the formation of esters. Branched-chain alcohols 2-methyl-1-propanol and 3-methyl-1-butanol come from reduction of branched-chain aldehydes and can be found in raw milk cheeses with intense proteolysis. The presence of these alcohols could be favoured in Castelo Branco cheese by the use of the highly proteolytic vegetable rennet. 3-Methyl butan-1-ol is also a major alcohol in La Serena cheese (Carbonell et al., 2002).

3.3.4. Esters

Most esters have floral and fruity notes and may contribute to cheese aroma by minimising the sharpness and bitterness imparted by fatty acids and amines, respectively. Higher content of esters was observed in MCB cheeses. Twelve esters were identified, however, only six were present in the three groups of cheeses, including, ethyl propanoate, propyl acetate, ethyl butanoate, 3-methylbutyl acetate, ethyl hexanoate and ethyl octanoate (Table 1). According to literature (Carbonell et al., 2002) ewe cheeses of higher quality present higher amounts of esters and free fatty acids.

3.3.5. Miscellaneous compounds

Hydrocarbons, coming from lipid oxidation have been frequently reported in the volatile fraction of ewe cheese (Carbonell et al., 2002). Seven hydrocarbons were identified, however, only 2-methylpentane and 3-methylpentane were present simultaneously in the three groups of cheeses (Table 2). Although the SPME/GC/MS method enables the extraction of terpenes, as observed previously (Pinho et al., 2004), no terpenes were identified in Castelo Branco cheeses, probably owing to the type of feed.

Eight aromatic compounds were identified in the three groups of cheeses; some of these compounds are described as products resulting from phenylalanine metabolism, namely benzoic acid, phenyl ethanol and 1-phenylpropan-2-one (Singh, Drake, & Cadwallader, 2003). Phenol and phenyl ethanol were present simultaneously in the three groups of cheeses (Table 2).

Six sulfur containing compounds were identified in the three groups of cheeses, these compounds are described as products resulting from methionine catabolism/or transamination (Singh et al., 2003). Methyldisulfanylmethane and methoxysulfonyloxymethane were identified in the three groups of cheeses and no significant differences were observed on the amount of those compounds (Table 2). ACB cheeses presented higher amounts of sulphur compounds and 3-methylbutanal. According to literature (Carbonell et al., 2002), ewe cheeses of lower quality contain higher amounts of sulphur compounds and 3-methylbutanal and cheeses of higher quality present lower concentrations of those compounds and higher concentrations of esters and free fatty acids.

Two alicyclic compounds, methylcyclopentane and 1-methyl-2-cyclohexene were identified in ACB cheeses.

Cheeses manufactured with milk from Merino and Assaf races and milk from crusade of these two races presented a total of 30 volatile compounds common, the remainder were different. The volatile compounds found in ‘‘Castelo Branco” cheese have been previously detected in other cheese varieties (Arques, Garde, Fernández-García, & Gaya, 2007; Barbieri et al., 1994; Engels, Dekker, de Jong, Neeter, & Visser, 1997; Fernández-Garcia, Carbonell, & Nuñez, 2002; Izco & Torre, 2000; Moio, Piombiono, & Addeo, 2000; Pinho et al., 2004; Sablé & Cottenceau, 1999; Tavaria et al., 2004; Urbach, 1993).

Analysis of cheeses volatile profile grouped by chemical classes highlighted that MCB cheeses presented all fatty acids and in general higher relative areas. Theses cheeses presented also higher abundance of esters. ACB cheeses presented poor composition of carbonyl compounds when compared with MCB and CCB, but ACB cheeses presented higher abundance of alcohols. In general, CCB cheeses presented an intermediate abundance of volatile when compared with MCB and ACB cheeses, indicating that the breed origin influenced cheese volatile profile.

Table 2
Aromatic, enxofre, alicyclic aldehyde and ester compounds identified by SPME–GC–MS analysis of the volatile fraction of ‘‘Castelo Branco” cheese according to ovine milk

Compounds	t_R (min)[A]	TIC/ion[B]	Relative areas achieved with different cheeses		
			MCB	ACB	CCB
Hydrocarbons					
Pentane	5.959	TIC	23^a	100^c	0^b
2-Methylbuta-1,3-diene	6.514	TIC	73^a	0^c	100^b
Pentane-1,3-diene	6.656	TIC	96^a	0^a	100^a
2,3-Dimethylbutane	7.672	TIC	100^a	79^c	0^b
2-Methylpentane	7.769	TIC	86^a	100^a	96^a
3-Methylpentane	8.263	TIC	67^a	100^b	55^a
Docecane	42.68	TIC	100^a	0^b	0^b
Aromatic					
Toluene	17.806	TIC	0^a	79^c	100^b
1,3-dimethylbenzene	24.264	TIC	0^a	100^b	87^b
Phenol	30.496	TIC	32^a	49^a	100^b
1-methyl-2-prop-2-enyl-benzene	37.271	TIC	100^a	0^b	0^b
2-Phenylethanol	38.494	TIC	60^a	100^b	71^a
1-Phenylpropan-2-one	39.299	TIC	75^a	100^a	0^b
Benzoic acid	40.316	TIC	100^a	0^b	0^b
Naphthalene	42.765	TIC	64^a	0^c	100^b
Sulphur					
Methylsulfanylmethane	6.533	TIC	100^a	0^b	0^b
1-Methylsulfanylethanone	13.607	TIC	0^a	100^c	70^b
Methyldisulfanylmethane	16.432	TIC	42^a	51^a	100^a
Methylsulfonylmethane	26.642	TIC	100^a	0^c	43^b
Methylsulfanyldisulfanylmethane	30.590	TIC	0^a	100^b	0^a
Methoxysulfonyloxymethane	35.526	TIC	100^a	91^a	95^a
Alicyclic					
Methylcyclopentane	23.886	TIC	0^a	100^b	0^a
1-Methyl-2-cyclohexene	33.917	TIC	0^a	100^b	0^a
Ether					
Ethoxyethane	6.167	59	0^a	100^c	63^c

[A] Retention time.
[B] When the resolution was not perfect chromatographic peak areas were not calculated from the ion current (TIC) but were estimated from integrations performed on the specified indicated ions.
[a,b,c] Means in lines without common letters are significantly different ($p < 0.05$).

3.4. Sensory analysis

The triangle test performed sixty-three non trained assessors revealed significant differences ($\alpha = 0.05$) between MCB and CCB cheeses, and between MCB and ACB cheeses concerning organoleptic characteristics. However, no significant differences were observed between ACB and CCB.

The mean results obtained by the 7 panellists for the 9 attributes assessed in MCB, CCB and ACB cheeses at 64 days of ripening are presented in Fig. 2. MBC cheeses presented highest classification of quality and intensity of odour and aroma. These cheeses, presented also higher scores for fatty aspect, sheep-like odour and rancid aroma. Respecting to acid and pungent aroma, similar scores were observed among MCB, CCB and ACB cheeses.

The results from quantitative descriptive analysis were in good agreement with cheeses volatile profile and fat content. MCB cheeses presented significantly higher fat content, associated with higher proportion of fatty acids, carbonyl compounds and esters (Fig 1). Especially, 2-methyl-propanoic, butanoic, 2-methylbutanoic, hexanoic and octanoic acids present in MCB cheeses in higher relative areas are associated with the attributes that showed highest scores in this cheeses (Table 2). Milk fat is important in artisanal cheese manufacturing (Tavaria et al., 2004). Replacement of whole milk with skim milk has proven that milk fat is an essential prerequisite for flavour development (Fox & Wallace, 1997).

The results from quantitative descriptive analysis were also in accordance with mean scores obtained for triangle test, indicating that non trained assessors could also distinguish MCB cheeses from others.

3.5. Discriminant analysis of volatile fraction of DOP "Castelo Branco cheese" to evaluate breed origin

Discriminant analysis was applied to data from volatile compound chromatographic areas. Two discriminant functions explained 100% of the total variance, function 1 explained 93.3% and function 2 explained 6.7%. Plots of the samples in functions 1 and 2 are presented in Fig 3. The discriminant analysis indicated that six variables contributed significantly to explaining the variability among the three types of cheese; ethyl acetate, butan-2-one, and 3-methylbutanoic acid were the major positive contributors to function 1 (loadings of 1.041, 0.813 and 0.728, respectively), and pentan-2-one was the major contributor with negative loading (−0.951). Pentan-2-ol, 3-methylbutyl acetate presented positive loadings for function 2 (0.932 and 0.751, respectively), pentan-2-one presented a negative loading in this function (−0.805).

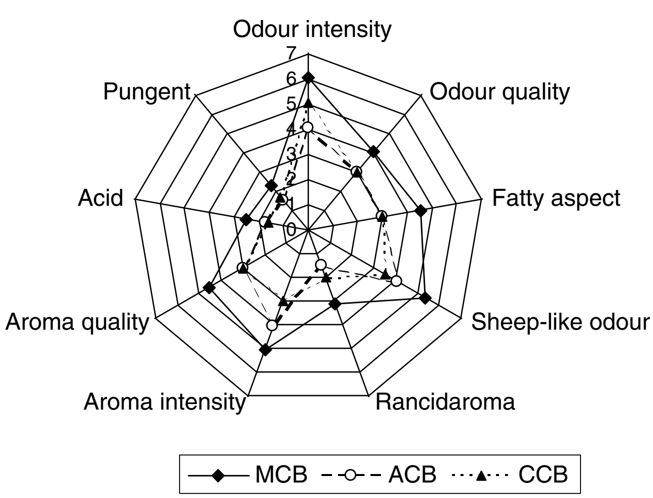

Fig. 2. Mean results for the nine sensory attributes assessed in MCB, ACB and CCB cheeses at 64 days of ripening.

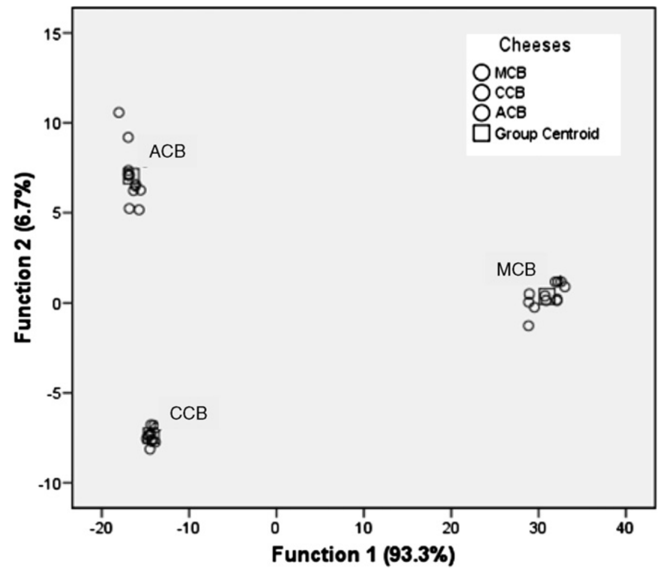

Fig. 3. Canonical discriminate functions showing MCB, ACB and CCB cheeses classified by the two functions of the proposed model.

Differences between MCB, ACB and CCB result from higher butan-2-one, ethyl acetate, 3-methylbutanoic acid abundance in MCB and higher abundance of pentan-2-ol and 3-methylbutyl acetate in ACB cheeses. The function thus obtained was able to correctly classify all the samples according to cheese type, indicating that discriminant analysis of volatile fraction of DOP "Castelo Branco cheese" can be used to evaluate breed origin.

4. Conclusions

Breeds from Merino da Beira Baixa, Assaf, and Crusade of these two races were grown under identical conditions. Lactating ewes were in similar physiological conditions. A similar diet was given to the three breeds before milk production. However, 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. More studies should be performed in other seasons to confirm these results, to establish the guidelines for production of high quality DOP "Castelo Branco" cheese.

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