International Dairy Journal 58 (2016) 23–30

Contents lists available at ScienceDirect

International Dairy Journal

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

Identification of odour-active compounds in ewes' raw milk commercial cheeses with sensory defects

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ARTICLE INFO

Article history:
Received 25 June 2015
Received in revised form
12 January 2016
Accepted 13 January 2016
Available online 28 January 2016

ABSTRACT

The aim of this work was to identify key odorant compounds associated with main off-flavours (acid, rancid and faecal) and one defect related to the internal appearance (big irregular eyes) in ewes' raw milk commercial cheeses. Cheese samples were submitted to solvent assisted flavour evaporation (SAFE) and odorant compounds were detected by gas chromatography–olfactometry (GC–O). Odour-active compounds detected by GC–O were identified and quantified by gas chromatography–mass spectrometry (GC–MS). Partial least square regression was performed to determine relationships between relative abundances of the odour-active compounds and sensory defects of commercial cheeses. An imbalance in the concentration of short-chain free fatty acids, predominant compounds in all cheese samples, was associated with acid and rancid off-flavour, whereas faecal off-flavour was related to minor compounds such as 4-methylphenol and 3-methyl-1-butanol. No volatile compound could be related to the defect of big irregular eyes.

1. Introduction

Flavour plays a key role in the overall quality of cheese and, therefore, the presence of sensory defects, and/or the lack of typical flavour, significantly decreases its quality causing financial losses for the dairy industry (Engel, Nicklaus, Septier, Salles, & Le Quéré, 2001). Cheese flavour is determined by the perception of a combination of large variety of volatile and non-volatile compounds in a particular balance (Le Quéré, 2004).

Volatile compounds are formed mainly during the ripening process. They are the result of microbial metabolism of residual lactose, lactate and citrate, formation of free fatty acids (FFAs), and casein degradation into a range of peptides and free amino acids (FAAs). The degradation of these compounds and the subsequent reactions between them also influences in the flavour composition (McSweeney & Sousa, 2000). Milk pasteurisation influences cheese volatile composition due to heat treatment inactivates enzymes, and reduces the amount of volatile compounds (some of them directly derived from feed) and native microorganisms of raw milk. Therefore, flavour tends to be stronger and more specific in raw milk cheeses than in pasteurised ones (Barron et al., 2007).

Although hundreds of volatile compounds have been identified in cheese, only few of them have been associated with its specific flavour (Curioni & Bosset, 2002). In many cases, the most abundant volatiles may have little odour impact and volatile compounds that are present at very low concentration contribute the most to cheese aroma. It is highly important to understand which compounds are key odorants because they can strongly determine the characteristic odour of the cheese (Smit, Smit, & Engels, 2005). Even though finding the volatile compounds responsible for aroma in dairy products is challenging, the study of compounds responsible for off-flavours could be appealing because each off-flavour often has a specific cause (Fox, McSweeney, Cogan, & Guinee, 2004; Thomsen et al., 2012).

Detection of key odorants is quite difficult because the sensibility of analytical instruments is lower than that of human nose perception (Curioni & Bosset, 2002). Gas chromatography–olfactometry (GC–O) has greatly improved the identification of odour-active compounds, in particular of those compounds responsible for off-flavours (Chamber & Koppel, 2013). Nevertheless, it should be emphasised that volatile compounds are analysed separately by GC–O and possible interactions between them might affect their

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http://dx.doi.org/10.1016/j.idairyj.2016.01.018
0958-6946/

perception. Consequently, this technique should be complemented with the sensory analysis of the food product (Chamber & Koppel, 2013; Thomsen et al., 2012).

Results from earlier studies (Zabaleta et al., 2016) indicated that sensory defects related with different kinds of openings in the cheese were the most frequently observed defects in ewes' raw milk commercial cheeses, and that some eye-related defects were associated with off-flavours. The objective of this study was to identify key odorant compounds associated with acid, rancid and faecal off-flavours, and one defect related to the internal appearance, big irregular eyes, in ewes' raw milk commercial cheeses.

2. Materials and methods

2.1. Commercial cheeses

Ewes's raw milk Idiazabal PDO cheeses were randomly collected from different producers for routine sensory quality control over three consecutive years. Cheeses were manufactured by cheesemakers according to the specifications of the Regulatory Council (Ministerio de Agricultura, Pesca y Alimentación, 1993). Briefly, raw milk was acidified with starter cultures and coagulated by addition of lamb rennet paste (or commercial rennet in some cases) at 28–32 °C. The coagulated milk was cut into rice-sized grains, stirred and heated to 35–37 °C for around 10 min. The curd was manually pressed in the vat and the whey removed. Then, the curd was distributed into cylindrical moulds, pressed about 6–8 h at approximately 20 °C, and placed in saturated sodium chloride brine at 10–12 °C for 16–24 h. Cheeses were ripened at 8–10 °C and around 85–90% relative air humidity for at least 2 months. The final cheese weighed approximately 1–2 kg.

A total of 752 Idiazabal cheeses were submitted to sensory quality control using the sensory analysis method described by Ojeda et al. (2015). Briefly, eight sensory parameters, cheese shape, rind, internal colour, eyes, odour, texture, flavour and persistence were scored by seven expert assessors on a 7-point discontinuous scale. In addition to positive sensory characteristics, sensory defects within each sensory parameter were assessed. Nine defects were selected due to their frequency of occurrence and/or their interest to the cheesemaking sector (acid, rancid, faecal, soft, adherent, pale, short cracks, big irregular eyes, and small rounded eyes; Zabaleta et al., 2016). Also, among all commercial cheeses submitted to sensory quality control, eight cheeses (two different cheeses for each sensory defect) were selected for gas-chromatography analysis because they showed only one of the four defects, namely, acid, rancid and faecal off-flavour, and big irregular eyes. The presence of a defect in the cheese was considered when at least six of the seven assessors indicated it in each session. In addition to these selected defective cheeses, two non-defective commercial cheeses were also selected as control cheeses. Cheeses were cut into 250 g triangular portions, vacuum packed in plastic bags, and frozen at −35 °C until physico-chemical analysis.

Ages of selected defective and non-defective cheeses ranged between 2 and 5 months. Average gross composition of these cheeses (mean ± standard deviation) was the following: dry matter (DM) percentage 65.36 ± 3.46, total fat and protein percentage on DM of 45.94 ± 6.70 and 35.77 ± 2.01, respectively, titratable acidity of 22.97 ± 3.17 mmol of sodium hydroxide per 100 g, and pH of 5.25 ± 0.15.

2.2. Extraction of volatile compounds

Selected defective and non-defective cheeses were submitted to solvent assisted flavour evaporation (SAFE) as previously described (Thomsen, Gourrat, Thomas-Danguin, & Guichard, 2014). The volatile compounds present in the resulting aqueous distillate were extracted with 40 mL of a 125 ng mL^{-1} solution of n-eicosane (internal standard; >99% purity, Sigma–Aldrich, Saint-Quentin Fallavier, France) in dichloromethane (99.9% purity, Carlo Erba Reagents, Val de Reuil, France). After drying, filtering and concentrating the organic phase, the extracted volume was adjusted to 400 μL with dichloromethane and stored at −20 °C for further analysis. Three replicate extracts containing volatile compounds were obtained from each cheese.

2.3. Gas chromatography–mass spectrometry

Volatile compounds present in each of the three replicate extracts obtained from each cheese were analysed by GC–MS by injecting (2 μL) of extract in splitless mode. Separation was performed using an Agilent 6890A gas chromatograph (Hewlett–Packard, Palo Alto, CA) coupled to a 5973 quadrupole mass spectrometer (Agilent, Palo Alto, CA). Volatile compounds were separated in a DBWAX fused silica capillary column (30 m length × 0.32 mm i.d. × 0.5 μm film thickness; Agilent J&W, Santa Clara, USA), using the following temperature gradient: oven temperature was held at 40 °C, then increased at a rate of 4 °C min^{-1} up to 240 °C and held for 10 min. Helium was used as carrier gas at 1.5 mL min^{-1}. Identification of volatile compounds was done by comparison of their experimental linear retention index (LRI), mass spectra with data from four databases: NIST 2.0 (National Institute of Standard and Technology, New York, USA), WILEY 138 library (Wiley & Sons Inc., New York, USA), INRAMass (Internal database achieved using standard compounds, INRA, Dijon, France) and Volatile Compounds in Food 15.2 (Zeist, The Netherlands). Limits of detection (LOD) and quantification (LOQ) were calculated as twice and four times the average noise (arbitrary units) respectively. Ten blanks (analysis of dichloromethane) were used to calculate the average noise in three different parts of the chromatogram. Volatile compound relative abundance (arbitrary units) in each cheese sample was calculated from the total ion current (TIC) multiplying by 100 the division between their peak area and that of internal standard (n-eicosane).

2.4. Gas chromatography–olfactometry

One cheese for each defect (acid, faecal and rancid off-flavour, and big irregular eyes appearance), and one non-defective cheese were selected for GC–O analysis. Three replicate extracts of each cheese were mixed and the resulting solution was analysed by GC–O by a panel of eight assessors, and each cheese extract was analysed in duplicate for each assessor. A total of 16 olfactometric determinations were done for each cheese extract. Odour zones (OZs) were detected using a HP 6890A GC (Agilent Technologies, Santa Clara, USA) coupled to two detection ports (split ratio 1:1); a flame ionisation detector (FID), and an olfactometric detection port (ODP). Chromatographic conditions were the same as for GC–MS analysis. The duration of olfactometric session was 40 min per cheese sample analysis. Each time the assessors detected an OZ, they pointed out one descriptor for the perceived odour using their own vocabulary. Odour descriptions and number of positive determinations for OZs were recorded using AcquiSniff® software (UR QuaPa/T2A; Saint Genès Champanelle, France). Only OZs with 5 or more positive determinations by the assessors for at least one cheese were considered.

2.5. Data treatment and statistical analysis

Three significant figures were used to express (mean and standard deviation) the relative abundance of volatile compounds.