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

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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 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 cheese-makers 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 $\pm$ 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 μ L) 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 $\times$ 0.32 mm i.d. $\times$ 0.5 μ m film thickness; Agilent J&W, Santa Clara, USA), using the following temperature gradient: oven temperature was held at 40 $^{\circ}$ C, then increased at a rate of 4 $^{\circ}$ C min $^{-1}$ up to 240 $^{\circ}$ 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. XLSTAT statistical software (version 2011.2, Addinsoft, France) was used for data treatment. One-way analysis of variance (ANOVA) was performed to determine significant differences ($P \leq 0.05$) in the relative

abundances of individual volatile compounds, and in the sum of relative abundances of all compounds of the same chemical family, between commercial cheeses with different sensory defects, including also non-defective commercial cheeses. Tukey's test was used for multiple comparisons between different cheeses. Partial least square regression (PLSR) was performed to determine relationships between relative abundances of the odour-active compounds and sensory defects of the cheeses. Only those odour-active compounds which showed scores for Variable Importance in the Projection (VIP) higher than 1, and loadings higher than 0.5 in the first two factors were included.

3. Results and discussion

3.1. Sensory defects in selected commercial cheeses

Fig. 1 shows the number of positive determinations for sensory defects in the selected commercial cheeses with acid, rancid and faecal off-flavour, big irregular eyes, and also in non-defective cheeses. This sensory analysis was made by the seven expert assessors panel on two different cheeses for each defect, and also for the non-defective samples. Acid off-flavour cheeses showed clearly other sensory defects such as pale colour and soft and adherent texture (positive determinations higher than 10). The other off-flavour cheeses did not significantly show other appearance and texture defects although short cracks and small rounded eyes were positively pointed out eight times in rancid and faecal off-flavour cheeses, respectively. Cheeses with big irregular eyes showed adherent texture, whereas the number of positive determinations for selected sensory defects in the non-defective cheeses was lower than four.

3.2. *Odour zones*

The assessors detected 72 different OZs by GC-O in the five tested cheeses. Most frequently cited odour descriptors and volatile compounds identified in each OZ are given in Table 1. One single volatile compound was identified by GC-MS in 55 OZs. These volatile compounds belonged to different chemical families, comprising acids, ketones, alcohols, esters, furans, lactones, aldehydes, phenolic compounds, pyrazines, sulphur compounds and terpenes.

Each of the five cheeses was described by GC-O with 52 to 63 different OZs but only 38 OZs were common for all the cheeses, suggesting that the corresponding volatile compounds were the main contributors to the overall cheese odour. Among these common OZs, strong odour notes like vinegar, cheesy, sweat, foot and dust were associated with n-butanoic and n-hexanoic acids and (E)-non-2-enal. It is well known that these compounds in the correct balance are major contributors to the characteristic odour of different types of cheeses (Bosset & Gauch, 1993; Fox et al., 2004; Hernández et al., 2009). Acids were the compounds with the highest relative abundance in the five tested cheeses (Table 2). Those predominant compounds contribute strongly to the aroma of Idiazabal PDO and other different cheese varieties such as, Manchego and Cheddar (Barron et al., 2005b; Frank, Owen, & Patterson, 2004). According to the high content of FFAs in all the cheeses, including non-defective ones (Table 2), lipolysis is a major pathway for flavour generation in this cheese variety. It has been reported that the use of lamb rennet paste which contains a potent pregastric lipase that liberates mainly short-chain fatty acids, gives a characteristic strong and piquant flavour (Amores et al.,

2013). Short-chain FFAs contribute to cheese flavour more than long-chain ones since they have a lower flavour threshold (Fox & Wallace, 1997; McSweeney & Sousa, 2000; Ziino, Condurso, Romeo, Giuffrida, & Verzera, 2005).

In addition, γ -hexalactone, γ -nonalactone, γ -decalactone and δ -decalactone, formed by intramolecular esterification of C4 or C5 hydroxyacids (Fox & Wallace, 1997; Smit et al., 2005), and esters such as ethyl butanoate, ethyl hexanoate and ethyl octanoate provided sweet odour notes (floral, fruity and coconut) to cheese aroma, as was also described in other cheese varieties (Frank et al., 2004; Moio, Dekimpe, Etiévant, & Addeo, 1993).

3.3. *Relationships between odour-active compounds and sensory defects*

PLSR analysis (Fig. 2) showed that the detected odour-active compounds were associated with most of the sensory defects reported in Fig. 1. The first two components (t1 and t2) explained 69.3% of the variability of odour-active compounds. Rancid off-flavour cheeses were separated from the others on the positive values of the first component, while the second component (t2) separated acid from faecal off-flavour cheeses. Cheeses with big irregular eyes and non-defective cheeses were not differentiated by PLSR analysis and could not be associated with any odorant compound. This seems logical because non-defective cheeses did not present any sensory defects and cheeses with big irregular eyes did not show any flavour-related defect (Fig. 1). In this sense, as discussed below for short-cracks and small rounded eyes, it seems that undesirable microbial growth would not be the cause for big irregular

eyes in cheese, and that mechanical reasons could be associated to this appearance defect.

3.3.1. Key odorants related to acid off-flavour

According to PLSR results, acid off-flavour, pale, adherent, and soft defects were related to acetic acid and tridecan-2-one (Fig. 2). These results confirmed the above mentioned sensory characterisation of acid off-flavour cheeses (Fig. 1). Acids were the most abundant volatile compounds in acid off-flavour cheeses although the content of which was not significantly different from the other cheeses, even lower than in rancid off-flavour cheeses (Table 2). Acid off-flavour of cheeses has been correlated with high concentration of lactic acid or low pH in cheese (Engel, Nicklaus, Salles, & Le Queré, 2002). However, no significant differences were found in titratable acidity and pH between cheeses with acid off-flavour and the other tested commercial cheeses. In this sense, other authors attributed this off-flavour to an excessive concentration of short-chain FFAs (McSweeney & Sousa, 2000) or to an excessive or unbalanced proteolysis during cheese ripening (Fox & Wallace, 1997).

Acetic acid, even though being low in relative abundance in comparison with other longer chain acids in tested commercial cheeses, was positively and highly correlated with acid off-flavour, and its relative abundance in acid off-flavour cheeses was significantly higher than in faecal off-flavour cheeses and non-defective cheeses (Table 3). Acetic acid was involved in the typical sharp-vinegar and acid odour notes and it has been considered an important flavour compound in Idiazabal PDO cheeses and other cheese varieties as Cheddar, Gruyère, Emmental, and Manchego (Barron et al., 2005b; Curioni & Bosset, 2002; Frank et al., 2004). In contrast to other acids, its

origin is not lipolytic because it is a result of citrate and lactic acid metabolism by lactic acid bacteria, or catabolism of Ala and Ser by starters and other bacteria (McSweeney & Sousa, 2000; Smit et al. 2005; Ziino et al., 2005).

Tridecan-2-one was also positively associated with acid off-flavour (Fig. 2). This medium-chain methyl ketone, which contributes with herb and spice odour notes (Volatile Compounds in Foods 16.1, 2015), has been found in very low concentrations in some blue cheese varieties (Gallois & Langlois, 1990, González de Llano, Ramos, Polo, Sanz, & Martínez-Castro, 1990). Some authors have reported that this type of methyl ketones could be formed by moulds from ketoacids naturally present in milk fat or by oxidation of monounsaturated fatty acids (Collins, McSweeney, & Wilkinson, 2003).

3.3.2. *Key odorants related to rancid off-flavour*

Rancid off-flavour was associated with 13 volatile compounds and short cracks defect according to PLSR results (Fig. 2). Short cracks defect was found in rancid off-flavour cheeses according to above mentioned sensory analysis (Fig. 1). Volatile compounds were seven linear saturated fatty acids, from n-butanoic acid to n-decanoic acid, which impart cheesy, sweat, and rancid odours, 4-methylpentanoic acid, described as herbal and cheesy, and n-dec-9-enoic acid, with herbal and flower aroma, three esters, ethyl hexanoate, ethyl decanoate and methyl decanoate, and γ -nonalactone, with flower, fruity, herbal, and coconut odour notes. Relative abundance of acids was significantly higher than in the other tested commercial cheeses (Table 2) mostly due to the high content of n-butanoic acid in rancid off-flavour cheeses (Table 3).

Rancidity in cheese has been attributed to an excessive or unbalanced lipolysis, which leads to an excess of FFAs producing off-flavours (Fox & Wallace, 1997; Fox et al., 2004; McSweeney & Sousa, 2000). n-Butanoic acid, present in significantly higher relative abundance in rancid off-flavour cheeses than in the other tested cheeses (Table 3), has often been described as a key odorant with cheesy or putrid odours (Barron et al., 2005b; Thomsen et al., 2012). High concentration of n-pentanoic acid has also been found in ewes' raw milk cheeses with intense lipolysis (Barron et al., 2005a). The high levels of n-butanoic and n-pentanoic acids found in rancid off-flavour cheeses (Table 3) together with the presence of short cracks defect could be associated to the late blowing defect of cheese (Gómez-Torres, Garde, Peirotén, & Ávila, 2015). FFAs are also precursors of other potent odorant compounds such as methylketones, lactones and esters (Fox & Wallace, 1997; McSweeney & Sousa, 2000; Smit et al., 2005).

3.3.3. *Key odorants related to faecal off-flavour*

Faecal off-flavour was related to small rounded eyes on the PLSR plot (Fig. 2), as observed in a previous study (Zabaleta et al., 2016). Faecal off-flavour cheeses showed significantly higher abundance of phenolic compounds than the other tested cheeses, and also significantly higher abundance of alcohols and aldehydes than non-defective cheeses (Table 2). PLSR analysis highlighted an association between faecal off-flavour and 2-phenylethanol, 4-methylphenol, 3-methyl-1-butanol, phenol and methionol (Fig. 2).

3-Methyl-1-butanol is generally considered as a result of Strecker degradations of Leu, forming 3-methyl-1-butanal that is rapidly reduced to 3-methyl-1-butanol by the activity of lactic acid bacteria dehydrogenases (Chamber & Koppel, 2013; Curioni &

Bosset, 2002; Fox & Wallace, 1997). Panel assessors gave descriptors such as burned and herbal to 3-methyl-1-butanol. Its odour has been described with descriptors such as dark chocolate, green, burned, faecal and sheep (Chamber & Koppel, 2013; Garde, Ávila, Medina, & Nuñez, 2005; McSweeney & Sousa, 2000; Morales, Fernández-García, Gaya, & Nuñez, 2003). Its relationship with unclean flavour and gassy openings was reported in Egyptian Ras cheese (Ayad, Awad, El Attar, de Jong, & El-Soda, 2004).

High concentration of phenolic compounds can exert a negative effect to cheese flavour with unpleasant odour notes (Curioni & Bosset, 2002). 4-Methylphenol (*p*-cresol) was present in significantly higher relative abundance in faecal off-flavour cheeses than in the other cheeses (Table 3). This compound is formed from Strecker degradation of Tyr, being a potent odorant that causes in cheese strong unclean flavours as putrid and strong medicinal aromas, and cowy odour notes (Curioni & Bosset, 2002; Frank et al., 2004; McSweeney & Sousa, 2000).

3.3.4. Key odorants related to non-defective cheeses

As mentioned above, PLSR analysis did not differ between cheeses with big irregular eyes and non-defective cheeses (Fig. 2). Acids were major volatile compounds in both types of cheese, followed by lactones and ketones (Table 2). The last two groups of compounds, even in low concentrations, contribute to the overall cheese aroma with flowery, apricot and coconut odour notes because they have very low perception thresholds (Curioni & Bosset, 2002).

4. Conclusions

Acids, esters, ketones and lactones were predominant volatile compounds in the ten tested commercial defective and non-defective cheeses. Those compounds, in appropriate concentrations, were involved in the basic cheese aroma of these ewes' raw milk commercial cheeses, but an imbalance in the concentration of them caused an unpleasant cheese flavour. This was observed for acids that, in high proportions, led to acid and/or rancid off-flavour, and for some minor odorant compounds such as 4-methylphenol and 3-methyl-1-butanol for faecal off-flavour. The results showed that both the quantification of odour-active compounds and their odour description are necessary to detect off-flavours in cheese. This study contributes to a better understanding of both the control of the sensory quality in ewes' raw milk commercial cheeses and the identification of key odorant compounds associated with off-flavours. Further studies including a higher number of cheeses are required to validate our results.

Acknowledgements

The authors would like to acknowledge the panel assessors. Financial support for this study was provided by the Basque Government (IT766-13), Department of Industry (SA-2010/00113), ChemoSens Platform, Burgundy Region and FEDER. Ms L. Zabaleta thanks a Predoctoral fellowship from the Department of Agriculture of the Basque Government.

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Table 1

Odour zones, main odour descriptors, number of positive determinations by GC-O (8 assessors $\times$ duplicate analyses), and volatile compounds identified by GC-MS in tested defective and non-defective commercial cheeses ^a

OZ	Odour descriptors	Number of positive determinations					LRI _(Exp)	LRI _(Lit)	DB	Volatile compounds
		A	B	C	D	E				
										Acids
1	Acid, bitter almond	11	8	15	0	0	1577	1576	1	2-methylpropanoic acid
2	Cheesy, fruity	13	13	16	6	13	1678	1677	3	3-methylbutanoic acid
3	Herbal, cheesy	3	9	2	3	2	1822	1820	3	4-methylpentanoic acid
4	Vinegar, acid	16	14	15	15	13	1457	1455	3	acetic acid
5	Cheesy, sweat	13	15	15	16	15	1628	1627	3	n-butanoic acid
6	Cheesy, burned	6	11	3	3	6	1752	1749	3	n-pentanoic acid
7	Cheesy, foot, faecal	15	15	16	16	13	1850	1853	3	n-hexanoic acid
8	Faecal, rancid	9	10	6	11	1	1968	1965	3	n-heptanoic acid
9	Faecal, dust, cleaner	15	13	15	15	13	2067	2070	1	n-octanoic acid
10	Faecal, burned, fruit	7	8	10	8	7	2184	2173	3	n-nonanoic acid
11	Dust, wax, burned	13	5	11	13	15	2282	2284	3	n-decanoic acid
12	Herbal, flower	0	6	0	1	0	2344	2344	1	n-dec-9-enoic acid
13	Chemical, cowshed	8	4	11	8	9	1948			unknown acid
										Ketones
14	Blue Cheesy, flower	1	2	1	0	13	1185	1184	4	heptan-2-one
15	Butter, flower	7	8	9	11	15	1291	1290	3	3-hydroxybutan-2-one
16	Flower, metal, herbal	15	10	15	14	16	1380	1395	3	1-hydroxybutan-2-one
17	Bread, vegetable	13	0	13	7	10	1817	1815	4	tridecan-2-one
18	Baked, celery	13	0	13	7	10	1447			unknown ketone
										Alcohols
19	Burned, herbal	15	10	15	14	16	1217	1229	3	3-methyl-1-butanol
20	Fruit, herbal	9	0	0	7	12	1330	1332	3	heptan-2-ol
21	Grilled, bread	13	0	13	7	10	1346	1340	3	2-methylpentan-3-ol
22	Fruity	0	0	0	0	11	1592	1566	4	butane-2,3-diol
23	Cookie, flower	1	2	1	0	13	1880	1869	4	phenylmethanol
24	Rose, violet, wine	7	8	9	11	15	1921	1905	4	2-phenylethanol
										Esters
25	Fruit, flower	0	5	0	0	4	1141	1165	3	butan-2-yl butanoate

26	Cabbage, garlic	0	3	3	0	6	1391	1389	3	3-hydroxy-2-butanone, acetate
27	Herbal, humidity	0	13	1	0	2	1603	1604	3	methyl decanoate
28	Fruit, olive	4	1	14	1	6	1071	1070	3	ethyl 3-methylbutanoate
29	Fruit, flower	15	15	14	13	13	1040	1037	3	ethyl butanoate
30	Flower, fruity	13	15	9	12	5	1240	1234	3	ethyl hexanoate
31	Burned, earth, flower	16	14	9	0	0	1444	1446	3	ethyl octanoate
32	Cheesy, fruity	0	7	0	0	6	1562	1553	1	ethyl nonanoate
33	Flower, humidity	4	4	6	0	8	1652	1652	4	ethyl decanoate
34	Flower, vanilla	2	9	5	15	9	1896	1870	4	ethyl dodecanoate
35	Peanut, grilled	0	0	0	0	2	1679	1671	3	ethyl benzoate
36	Flower, fruity	2	11	10	1	3	1429	1421	3	butyl hexanoate
Furans										
37	Chlorine, cleaner	2	0	3	1	6	1620	1616	3	5-methyloxolan-2-one
38	Roasted, fruity	6	6	3	0	6	1887		2	2,5-dimethoxy-2,5-dihydrofuran
Lactones										
39	Baked, vegetable	8	6	13	8	12	1709	1696	3	γ -hexalactone
40	Coconut, fruit, spice	7	9	11	0	13	1980	1977	3	δ -octalactone
41	Cotton candy, coconut, caramel	14	6	6	14	13	2035	2024	3	γ -nonalactone
42	Fruit, apricot, flower	6	5	10	8	5	2154	2173	3	γ -decalactone
43	Peach, celery, flower	11	13	8	14	13	2205	2209	3	δ -decalactone
44	Green, fruity	2	0	3	0	8	2382	2384	3	γ -dodecalactone
45	White flowers, orange	4	5	13	9	7	2233			unknown lactone
Aldehydes										
46	Herbal	4	1	9	3	7	1089	1091	3	n-hexanal
47	Peas, plastic	0	1	3	0	9	1412	1409	3	n-nonanal
48	Chemical, faecal	8	1	1	5	5	2134	2146	3	n-hexadecanal
49	Vegetable, grass	0	6	15	3	0	1438	1434	4	(E)-oct-2-enal
50	Carboard, dust	10	8	15	13	13	1542	1543	4	(E)-non-2-enal
51	Grilled, sweat	0	1	8	4	4	1772	1777	4	(2E,4E)-deca-2,4-dienal
Phenolic compounds										
52	Metal, smoke	4	3	5	6	9	2012	2003	4	phenol
53	Faecal, cowshed	13	4	12	11	13	2088	2068	3	4-methylphenol
54	Sheep, grilled	8	0	10	10	5	2097	2090	3	3-methylphenol

55	Bitter almond, roasted potato	6	0	0	0	0	1414	1391	4	Pyrazines 2,3,5-trimethylpyrazine
56	Potato	11	12	0	15	3	1473	1453	4	Sulphur compounds methional
57	Anis, chlorophylle, flower	13	6	14	12	11	1725	1714	4	methionol
58	Grass	3	3	2	6	5	1206	1210	3	Terpenes d-limonene
59	Penetrant, vegetable	2	6	1	0	0	1198			Unknown compounds
60	Grilled, vegetable	8	0	0	0	10	1247			
61	Mushroom	15	13	15	15	15	1308			
62	Flower, herbal	15	11	16	14	15	1592			
63	Toasted, vegetable, flower	2	0	0	6	0	1759			
64	Flower, herbal	9	1	4	2	1	1779			
65	Candy, flower	6	5	7	10	8	1869			
66	Purin, solvent	0	2	0	0	9	2122			
67	Spice, cumin	2	6	2	1	1	2268			
68	Fruit, coconut	8	9	12	9	0	2390			
69	Herbal, chemical	7	0	13	10	11	1513			
70	Coconut, candy, sweet	12	8	7	13	10	1927			
71	Fruity, flower	0	7	8	0	5	2110			
72	Faecal, chemical	3	11	11	7	0	2276			

^a 1 cheese of each type of cheese A, B, C, D and E was analysed: A, acid off-flavour cheese; B, rancid off-flavour cheese; C, faecal off-flavour cheese; D, cheese with big irregular eyes; E, non-defective cheese. Abbreviations are: OZ, odour zone number; LRI_(exp), experimental linear retention index; LRI_(lit), linear retention index reported in the literature on DB-WAX column. DB, database: 1, NIST 2.0; 2, WILEY 138 library; 3, INRAMass; 4, Volatile Compounds in Food 16.1.

Table 2Relative abundance of odour-active compounds grouped by chemical families in tested defective and non-defective commercial cheeses. ^a

	Cheeses				
	A	B	C	D	E
Acids	16500±1900 ^b	35000±10200 ^a	9010±2330 ^b	10400±2420 ^b	10300±5830 ^b
Ketones	361±77.4 ^b	991±1040 ^{ab}	300±68.0 ^b	954±568 ^{ab}	1530±397 ^a
Alcohols	154±91.5 ^b	782±791 ^{ab}	1090±103 ^a	717±465 ^{ab}	265±14.6 ^b
Esters	795±97.1 ^a	605±285 ^a	248±195 ^a	589±676 ^a	362±76.6 ^a
Furans	4.44±4.24 ^a	13.9±9.63 ^a	2.68±0.813 ^a	5.72±2.97 ^a	324±893 ^a
Lactones	203±44.0 ^a	215±87.8 ^a	211±34.1 ^a	1720±2140 ^a	575±39.6 ^a
Aldehydes	71.4±53.7 ^{ab}	59.5±42.1 ^{ab}	191±171 ^a	22.8±3.45 ^b	87.9±17.5 ^b
Phenolic compounds	18.4±14.9 ^b	18.0±5.92 ^b	88.1±16.1 ^a	15.7±1.59 ^b	9.12±0.456 ^b
Pyrazines	1.69±0.949 ^b	ND	6.66±6.96 ^b	62.8±51.3 ^a	18.5±9.59 ^b
Sulphur compounds	2.73±0.656 ^b	5.90±0.833 ^{ab}	8.02±4.19 ^a	4.97±1.15 ^{ab}	7.97±1.59 ^a
Terpenes	1.13±0.607 ^{ab}	1.02±0.256 ^b	0.710±0.199 ^b	1.10±0.488 ^{ab}	1.81±0.553 ^a

^a Values (in arbitrary units; mean ± standard deviation) are data from 2 cheeses × 3 replicate GC-MS analyses for each type of cheese A, B, C, D and E: A, acid off-flavour cheeses; B, rancid off-flavour cheeses; C, faecal off-flavour cheeses; D, cheeses with big irregular eyes; E, non-defective cheeses. Means followed by a different superscript letter in the same row are significantly different ($P \leq 0.05$); ND, peak below limit of detection.

Table 3

Relative abundance of key odorant compounds associated with acid, rancid and animal off-flavour in tested defective and non-defective commercial cheeses.^a

Key odorant compounds	Relative abundance				
	A	B	C	D	E
Acid off-flavour					
n-Acetic acid	260±65.4 ^a	261±71.2 ^a	39.1±33.0 ^b	170±89.5 ^{ab}	49.3±24.4 ^b
Tridecan-2-one	51.9±31.2 ^a	ND	3.62±4.29 ^a	7.53±3.41 ^a	6.10±6.71 ^a
Rancid off-flavour					
4-Methylpentanoic acid	1.28±1.74 ^b	66.3±21.0 ^a	10.2±6.57 ^b	0.762±0.866 ^b	4.76±2.57 ^b
n-Butanoic acid	2130±241 ^b	7620±2130 ^a	725±249 ^b	1820±323 ^b	1720±959 ^b
n-Pentanoic acid	55.9±23.0 ^a	751±468 ^a	24.4±8.82 ^a	55.3±16.5 ^a	101±88.6 ^a
n-Hexanoic acid	4070±646 ^{ab}	10900±3270 ^a	1940±678 ^b	2690±545 ^{ab}	3060±1930 ^{ab}
n-Heptanoic acid	251±65.7 ^a	745±385 ^a	116±45.0 ^a	126±67.7 ^a	138±90.3 ^a
n-Octanoic acid	4110±805 ^a	7620±2280 ^a	2590±1030 ^a	2170±437 ^a	2560±1480 ^a
n-Nonanoic acid	264±128 ^a	233±101 ^a	91.9±35.4 ^a	53.8±6.61 ^a	74.0±47.4 ^a
n-Decanoic acid	4190 ¹ ±546 ^a	5670±1760 ^a	2440±552 ^a	2380±297 ^a	2230±1050 ^a
n-Dec-9-enoic acid	306±157 ^{ab}	738±181 ^a	161±112 ^b	64.7±13.2 ^b	198±172 ^b
Ethyl hexanoate	85.4±46.2 ^a	261±153 ^a	42.4±23.2 ^a	33.6±3.70 ^a	25.9±8.08 ^a
Ethyl decanoate	104±88.1 ^a	203±71.0 ^a	106±81.7 ^a	38.8±27.3 ^a	31.8±16.7 ^a
Methyl decanoate	0.492±0.113 ^a	7.98±7.54 ^a	1.66±1.03 ^a	0.309±0.351 ^a	0.610±0.518 ^a
γ-Nonalactone	11.2±3.62 ^a	79.1±83.1 ^a	9.55±3.23 ^a	8.74±1.63 ^a	9.04±1.38 ^a
Faecal off-flavour					
2-Phenylethanol	67.9±44.6 ^b	44.7±38.4 ^b	486±69.1 ^a	42.0±33.3 ^b	5.14±5.93 ^b
3-Methyl-1-butanol	19.5±5.37 ^b	14.1±10.7 ^b	523±122 ^a	17.0±8.08 ^b	8.84±3.89 ^b
Phenol	ND	2.81±0.535 ^a	4.83±3.27 ^a	2.23±1.22 ^a	0.888±0.990 ^a
4-Methylphenol	11.9±8.48 ^b	5.87±2.34 ^b	63.1±28.0 ^a	8.61±1.49 ^b	4.57±1.21 ^b
Methionol	0.453±0.110 ^a	1.39±0.658 ^a	5.89±3.82 ^a	0.807±0.322 ^a	0.248±0.0404 ^a

^a Values (in arbitrary units; mean ± standard deviation) are data from 2 cheeses × 3 replicate GC-MS analyses for each type of cheese A, B, C, D and E: A, acid off-flavour cheeses; B, rancid off-flavour cheeses; C, faecal off-flavour cheeses; D, cheeses with big irregular eyes; E, non-defective cheeses. Means followed by a different superscript letter in the same row are significantly different ($P \leq 0.05$); ND, peak below limit of detection. Odour zone number and linear retention index are as given in Table 1.

Figure legends

Fig. 1. Number of positive determinations for sensory defects pointed out by the seven expert assessors panel from the sensory quality control of selected commercial cheeses. Each assessor evaluated two cheeses in different sessions for each type of cheese A, B, C, D and E. A (solid black line), acid off-flavour cheeses; B (dashed black line), rancid off-flavour cheeses; C (dashed grey line), faecal off-flavour cheeses; D (solid grey line), cheeses with big irregular eyes; E (grey line marked with ×), non-defective cheeses.

Fig. 2. Partial least square regression (PLSR) plot for relative abundances of odour-active compounds and sensory defects from the sensory quality control of selected commercial cheeses. Explained variance was 69.3% for odour-active compounds and 48.7% for sensory defects. Sensory defects are bold faced, odour-active compounds are numbered by OZ number as in Table 1, and cheese samples are marked in grey. A, acid off-flavour cheeses; B, rancid off-flavour cheeses; C, faecal off-flavour cheeses; D, cheeses with big irregular eyes; E, non-defective cheeses.

Figure 1

Figure 2