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Relationships between volatile compounds and odour in Manchego cheese: comparison between artisanal and industrial cheeses at different ripening times

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Abstract – Manchego cheese, produced from local ewe's milk in the La Mancha region of Spain under a Protected Designation of Origin (PDO), is Spain's most popular cheese variety and may be made from raw milk (artisanal cheese) or pasteurised milk (industrial cheese). As a consequence, the cheeses so manufactured may show differences in their organoleptic properties, associated with differences in volatile composition, important for cheese aroma. Samples of Manchego cheese made from raw milk and from pasteurised milk and aged for 2, 4, 6 and 12 months were analysed in this study of the volatile composition of the different cheese types, performed using two different analytical methods, Simultaneous Distillation Extraction (SDE) and Automatic Dynamic Headspace (P&T). Approximately 79 compounds were detected by SDE and 44 by headspace, falling in the chemical groups free fatty acids (FFAs), ketones, esters, alcohols and others. Also, olfactory profiles of the cheese samples were assessed by sensory analysis. Nine odour attributes (odour intensity, milk odour, lactic/yoghurt odour, briny odour, rancid odour, butter odour, dry hay odour, fruity odour and pungent sensation) were assessed sensorially. Both the concentrations of the volatile compounds detected and the intensity of the sensory odour attributes evaluated were found to vary during ripening. The results were distinctly different for the artisanal and industrial Manchego cheese samples.

Manchego cheese / ripening / volatile compound / sensory analysis

Résumé – **Relation entre les composés volatils et l'arôme dans les fromages Manchego artisanaux et industriels.** Le fromage Manchego d'Appellation d'Origine Protégée (AOP) est la variété la plus connue en Espagne. Sa production est limitée à la région de la Mancha. Ce fromage est fabriqué

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avec du lait de brebis, à partir de lait cru (fromage artisanal) ou pasteurisé (fromage industriel). En conséquence, les fromages peuvent présenter des différences dans leurs propriétés sensorielles, associées à des différences de composition en volatils, particulièrement déterminants pour l'arôme des fromages. Dans cette étude, nous avons analysé des fromages Manchego affinés pendant 2, 4, 6 et 12 mois, et fabriqués avec deux types de lait différents : cru et pasteurisé. Nous avons étudié la composition en volatils avec deux techniques analytiques différentes : Simultaneous Distillation Extraction (SDE) et Automatic Dynamic Headspace (P&T). Nous avons analysé le profil olfactif des fromages par analyse sensorielle. Environ 79 composés volatils ont été détectés par la technique SDE et 44 par P&T. Ils appartiennent à différentes familles chimiques : acides gras libres, cétones, esters, alcools et autres. Neuf descripteurs olfactifs ont été évalués par analyse sensorielle : intensité de l'odeur, odeur de lait, odeur lactique/yoghourt, odeur de saumure, odeur rance, odeur de beurre, odeur de foin, odeur fruitée et la sensation piquante. Les résultats ont montré que les concentrations en composés volatils et les intensités des descripteurs évalués, ont changé pendant l'affinage des fromages et ont été différents entre les fromages Manchego artisanaux et industriels.

Fromage Manchego / affinage / composé volatil / analyse sensorielle

1. INTRODUCTION

Manchego cheese is perhaps the most popular cheese variety in Spain. It is made in the La Mancha region from locally produced ewe's milk under a Protected Designation of Origin (PDO). The cheese can have a weight of between 1 and 3.5 kg. The PDO Regulatory Board has authorised two basic manufacturing processes, one artisanal and the other industrial. The former is carried out according to traditional usages, including enzymatic coagulation of raw ewe's milk, cutting, pressing, salting and ripening for at least two months, although the most prized cheeses are aged longer. The industrial process uses pasteurised milk, and hence a starter culture is always added before coagulation; the remaining steps are similar to those for the artisanal Manchego cheese. The cheeses so prepared can thus be expected to have different organoleptic properties. It is know that the pasteurisation has an effect on the biochemical modifications which occur during cheese ripening, and ultimately on the sensory characteristics of the cheeses (aroma, flavour and texture) [17]. A number of studies have been carried out on different aspects of Manchego cheese [3, 13, 14, 25, 33, 36]. Volatile components have also been examined, e.g. by Charro et al. [5], Martínez-Castro et al. [26] and Villaseñor et al. [44]. Despite the importance of a portion of the volatiles in producing the characteristic odour of Manchego cheese, little is known about the relative contribution of the different volatile components to aroma. Recently, Fernández García et al. [10] have studied the evolution of the volatile fraction in Manchego cheese manufactured with raw and pasteurised milk. They found high concentrations of aldehydes, esters and alcohols in both types of cheeses. Cheese aroma is a very complex perception and is the outcome of the action of many compounds that may also be found in other foods of different kinds [42], the balance among them being responsible for determining the typical aroma of a food. Sensory analysis is often used as a means of quality control and classification of food and beverages [6] and it has been an essential tool for determining the sensory character changes in cheeses over the ripening time [16]. However, some work dealing with the sensory characteristics of Spanish ovine cheeses has been performed [15, 34]. González Viñas et al. [16] have described the physicochemical, proteolysis and sensory and texture characteristics of Manchego cheeses during ripening. The data gathered in this study indicate that sensory and instrumental analysis are the most important tools for detecting changes in Manchego cheeses during ripening.

The object of this paper is therefore to attempt to compare the volatile composition with sensory data during ripening for genuine Manchego cheeses made according to the two basic authorised manufacturing processes indicated above, that is, artisanal and industrial cheeses, and also to study the influence of ripening time on volatile composition and on the sensory characteristics of both types of cheese.

2. MATERIALS AND METHODS

2.1. Samples

A total of eight samples (weighing between 1 and 2 kg) of Manchego cheeses made in winter from raw milk (artisanal cheeses) and pasteurised milk (industrial cheeses) ripened for 2, 4, 6 and 12 months were purchased directly from the manufacturers for analysis. All samples were produced by authorised manufacturers operating under the Manchego Cheese Protected Designation of Origin. No significant differences were recorded for the physicochemical parameters considered (a_w, pH, dry matter, NaCl and total nitrogen) between artisanal and industrial cheeses (results not shown). The values obtained for these parameters are in agreement with those obtained by other authors for Manchego cheeses [13, 16].

2.2. Volatile composition

Simultaneous distillation extraction (SDE) was carried out as previously [26], with pentane as extracting solvent and camphor as the internal standard. Samples were injected on an Autosystem gas chromatograph (Perkin-Elmer, Norwalk, CT, USA) using a Supelcowax capillary column (50 m × 0.25 mm × 0.25 μm d_f) using nitrogen as the carrier gas with a linear velocity of 9.1 $m \cdot min^{-1}$ and split flow of 55 $mL \cdot min^{-1}$. The injector temperature was 250 ºC. The oven temperature was programmed from 60 to 190 ºC at 4 $ºC \cdot min^{-1}$ and then held for 50 min. Data acquisition was performed with the program Chrom-Card for Windows v. 1.2 from Thermo Quest. Semiquantitative values ($\mu g \cdot g^{-1}$) were calculated from peak areas of volatile compounds and the internal standard obtained with the flame ionisation detector (FID). SDE extracts were also analysed by GC-MS.

Automatic dynamic headspace (P&T) was carried out as described previously [43], with 2-hexanone as the internal standard, using the same column mentioned above, and a Hewlett-Packard purge and trap concentrator (Avondale, PA), coupled to a GC-MS 5890 Hewlett-Packard system. Semiquantitative values ($\mu g \cdot g^{-1}$) were calculated from the total ion current areas of volatile compounds and the internal standard obtained with the mass detector.

MS data were acquired and processed by a HP-Chemstation. Identification was carried out from retention times and mass spectral data from the Wiley Library [27] and confirmed by using the retention times of standard compounds when available.

Since the aim of these analyses was to compare samples, differences in the response factor and recovery were not taken into account.

2.3. Descriptive sensory analysis

Sensory analysis sessions were carried out in a tasting room in individual booths equipped in accordance with the recommendations set out in ISO standard 8589 [19] at morning sessions (12:00 noon – 2:00 pm). The sensory analysis was performed by 11 assessors with previous experience in the sensory evaluation of cheeses, four men and seven women ranging between 25 and 40 years of age.

2.3.1. Training

The panellists underwent training. First, a broad list of attributes to describe the olfactory characteristics of the cheeses was generated, and the most frequently used descriptors were selected by consensus, eliminating synonyms. The intensity of the attributes thus selected was then evaluated using unstructured scales 10 cm long. Training was conducted for three weeks, using samples of commercial Manchego cheeses from stores, Manchego cheeses purchased directly from the manufacturers (the same cheeses used in this study) and some reference substances [2]. The cheeses were stored at the same conditions as in manufacture industry (7 ºC; 75% relative humidity) during the three weeks of training.

2.3.2. Olfactory profile

After panellist training, artisanal and industrial Manchego cheese samples were assessed in duplicate using unstructured scales. Sensory analysis of the samples took place during four sessions held over the course of one week. Four samples were served at each session, with all samples being served two times per panellist and in all places in order over the sessions.

Samples were presented to the assessors in the form of cubes measuring 1.5 cm per side at 20 ºC served on odour-free plastic plates, both during the training sessions and during the sensory analysis of the artisanal and industrial Manchego cheeses. All samples were identified by a 3-character code.

2.4. Statistical analysis

A two-factor analysis of variance (samples and session) was run on the sensory analysis data by assessor and by attribute in order to evaluate the assessors' discriminating ability (F_{sample}) and the reproducibility of the assessments ($F_{session}$). To evaluate the significance of differences between samples according to ripening time for each type of cheeses, the Student-Newman-Keuls test for comparison of means was applied. Also, the Student's-T test was used to evaluate the differences between samples according to the processing method for the same ripening time.

All statistical procedures were performed using the SPSS for windows, version 9.0 (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1. Descriptive sensory analysis

A total of nine descriptors for Manchego cheese odour were selected from all the terms generated by the taste panellists (odour intensity, milk odour, lactic/yoghurt odour, briny odour, rancid odour, butter odour, dry hay odour, fruity odour and pungent sensation).

3.1.1. Assessor discriminating ability and reproducibility

The two-factor analysis of variance showed that all the assessors exhibited a suitable level of discriminating ability, in that the F_{sample} values were significant ($P \leq 0.3$) for all assessors for all the attributes evaluated [39]. The analysis results for reproducibility also indicated that on the whole responses by individual assessors were reproducible, in that in most cases the value of $F_{session}$ was not significant ($P \geq 0.05$).

On the basis of the results all 11 panellists were deemed capable of assessing the odour of Manchego cheeses.

3.1.2. Olfactory profile

The mean scores awarded by the assessors to each of the attributes evaluated in the artisanal and industrial cheese samples are shown in Table I.

In the artisanal cheese samples, the changes in the olfactory characteristics became considerably more significant after six months of ripening, for most of the attributes assessed. The artisanal cheese samples aged for 12 months exhibited sensory characteristics that were significantly different from those of the rest of the samples ripened for shorter times. In contrast, in the case of the industrial cheese samples, the olfactory characteristics were quite similar whatever the ripening time.

A comparison of the sensory odour characteristics of the artisanal cheeses with those of the industrial cheeses for the same ripening times revealed significant differences

Table I. Mean values and standard deviation of the odour attributes evaluated in artisanal and industrial Manchego cheese and significant differences.

Cheeses	Ripening time			
Artisanal	2 months ($n = 2$)	4 months ($n = 2$)	6 months ($n = 2$)	12 months ($n = 2$)
Intensity	$6.6^{a} \pm 0.09$	$7.2^{b} \pm 0.01$	$6.1^{c} \pm 0.27$	$8.6^{d} \pm 0.04$
Milk	$3.5^{a} \pm 0.03$	$1.5^{b} \pm 0.23$	$1.1^{c} \pm 0.09$	$0.0^{d} \pm 0.00$
Lactic/yoghurt	$4.2^{a} \pm 0.33$	$3.7^{b} \pm 0.11$	$3.3^{b} \pm 0.12$	$0.0^{c} \pm 0.00$
Brine	$4.4^{ab} \pm 0.41$	$5.4^{b} \pm 0.60$	$3.7^{a} \pm 0.22$	$5.9^{b} \pm 0.42$
Rancid	$4.1^{a} \pm 0.71$	$5.7^{ab} \pm 0.61$	$5.1^{ab} \pm 0.44$	$6.6^{b} \pm 0.18$
Butter	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Pungent	$3.5^{a} \pm 0.52$	$5.2^{b} \pm 0.17$	$3.8^{a} \pm 0.34$	$7.2^{c} \pm 0.17$
Dry hay	$4.6^{a} \pm 0.13$	$4.1^{b} \pm 0.01$	$3.0^{c} \pm 0.20$	$5.7^{d} \pm 0.09$
Fruity	$2.8^{a} \pm 0.39$	$1.9^{b} \pm 0.19$	$2.3^{ab} \pm 0.13$	$0.0^{c} \pm 0.00$
Industrial				
Intensity	3.9 ± 0.37	4.0 ± 0.13	4.4 ± 0.49	4.6 ± 0.39
Milk	$4.3^{ab} \pm 0.24$	$4.4^{ab} \pm 0.15$	$4.6^{b} \pm 0.06$	$3.9^{a} \pm 0.12$
Lactic/yoghurt	$3.1^{a} \pm 0.01$	$3.7^{a} \pm 0.01$	$3.4^{a} \pm 0.20$	$2.6^{b} \pm 0.42$
Brine	1.9 ± 0.13	1.9 ± 0.07	1.8 ± 0.51	1.9 ± 0.36
Rancid	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Butter	$4.2^{a} \pm 0.43$	$5.2^{a} \pm 0.35$	$4.4^{a} \pm 0.19$	$3.8^{b} \pm 0.01$
Pungent	$0.0^{a} \pm 0.00$	$0.0^{a} \pm 0.00$	$0.0^{a} \pm 0.00$	$0.8^{b} \pm 0.18$
Dry hay	$0.0^{a} \pm 0.00$	$0.0^{a} \pm 0.00$	$1.9^{b} \pm 0.13$	$1.6^{b} \pm 0.20$
Fruity	$2.7^{a} \pm 0.11$	$3.1^{ab} \pm 0.11$	$3.5^{b} \pm 0.41$	$2.4^{a} \pm 0.12$

a–d: Means within rows without a common superscript are significantly different ($P \leq 0.05$) according to the Student-Newman-Keuls test.

between the cheese samples made by these two different manufacturing methods at all the ripening times considered (2, 4, 6 and 12 months) for all the attributes assessed ($t \geq 6.3137$; $P \leq 0.05$), the sole exception being the attribute lactic/yoghurt odour in the cheeses ripened for four and six months.

Fruity odour intensity was scored the same in both the artisanal and industrial cheese samples at two months of ripening. The scores for the attribute butter odour were relatively high in the industrial cheeses, whereas that odour attribute was not perceived in the artisanal cheeses. Furthermore, the artisanal cheese samples were awarded high scores for the attributes pungent odour and dry hay odour. Those attributes were undetected by the assessors in the industrial cheeses in the first months of ripening.

3.2. Volatiles

Figure 1 depicts the chromatographic profiles for the SDE extracts of 12-month-old artisanal (a) and industrial (b) cheeses, and Figure 2 presents the corresponding reconstructed chromatograms for the same cheeses analysed by P&T-GC-MS. About 79 volatile compounds were detected using SDE and 44 by dynamic headspace, these being classified in different chemical groups, namely, free fatty acids (FFAs), ketones, esters, alcohols and others. The ratios of these components varied during ripening and were distinctly different between the artisanal and industrial cheese samples.

Tables II and III list the compounds detected in both types of cheese using SDE and P&T.

Figure 3 (a and b) depicts the trends for the main groups of compounds found in both types of cheese. The FFAs behaved differently during the ripening period in the artisanal and industrial cheeses. In the artisanal cheeses FFA levels increased over the entire course of ripening; on the other hand, in the industrial cheeses, peak FFA levels were attained after four months of ripening and subsequently declined. At the end of ripening, FFA values were much higher in the artisanal cheese samples than in the industrial cheese samples. Ketones, esters and alcohols all followed similar trends.

SDE identified FFAs from butyric acid to dodecanoic acid. High levels of FFA have been found in hard cheeses such as Parmesan [4] and Grana Padano [29]. As has previously been reported [26], decanoic acid was the main acid in the artisanal cheeses, though a portion of the butyric acid remained dissolved in the aqueous phase, such fractionation being inherent in all processes of volatile isolation [1]. FFAs are important for the aroma of Manchego cheese not only in themselves but also as precursors of methylketones, alcohols and esters. It has been demonstrated that some FFAs (especially short-chain FFAs) contribute directly to the aroma characteristics of many types of cheese, or indirectly as precursors of the aroma components [11]. Lipolysis in cheeses like Cheddar or Manchego is not very intense in quantitative terms, but it is qualitatively important since it plays a major role in the development of the aroma characteristics of these varieties of cheese [9, 22].

Odd-chain methylketones of from 3 to 15 carbon atoms and some even-chain methylketones were found, the most abundant being 2-heptanone and 2-nonanone; nonen-2-one was also detected. High quantities of these compounds are usually present in mold-ripened cheeses, but they are also present in most hard and semi-hard varieties, such as Cheddar [24] and Gruyère de Comté [18]. Diacetyl and acetoin were very abundant in the industrial cheeses; these two compounds can be derived from citrate by different lactic acid bacteria, especially *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis*. High quantities of diacetyl give buttery notes to Hispanico

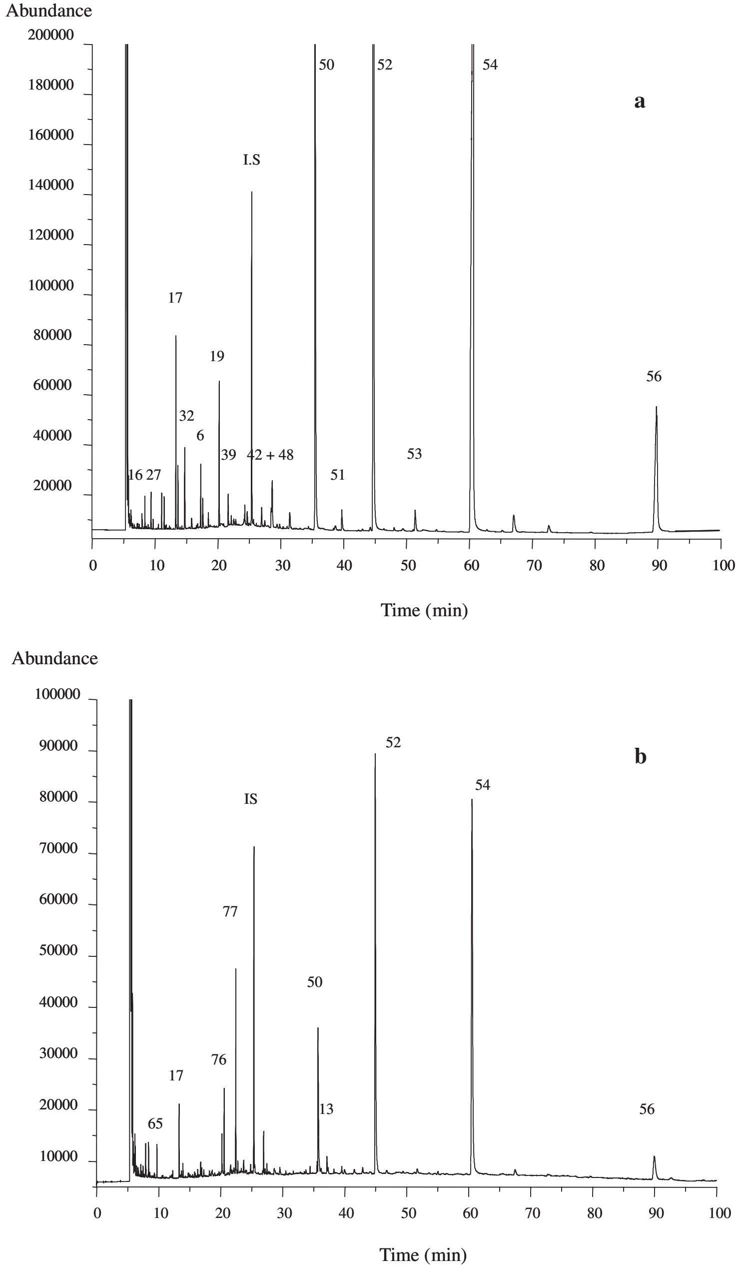

Figure 1. Different volatile compounds found by SDE at 12 months in artisanal cheese (a) and in industrial cheese (b).
(a): 2-pentanone (16), ethyl butyrate (27), 2-heptanone (17), ethyl hexanoate (32), 2-heptanol (6), 2-nonanone (19), ethyl octanoate (39), camphor (I.S), ethyl decanoate + butyric acid (42 + 48), hexanoic acid (50), heptanoic acid (51), octanoic acid (52), nonanoic acid (53), decanoic acid (54), dodecanoic acid (56);
(b): toluene (65), 2-heptanone (17), 2-butoxyethanol (76), butylglycol acetate (77), camphor (I.S), hexanoic acid (50), benzenemethanol (13), octanoic acid (52), decanoic acid (54), dodecanoic acid (56).

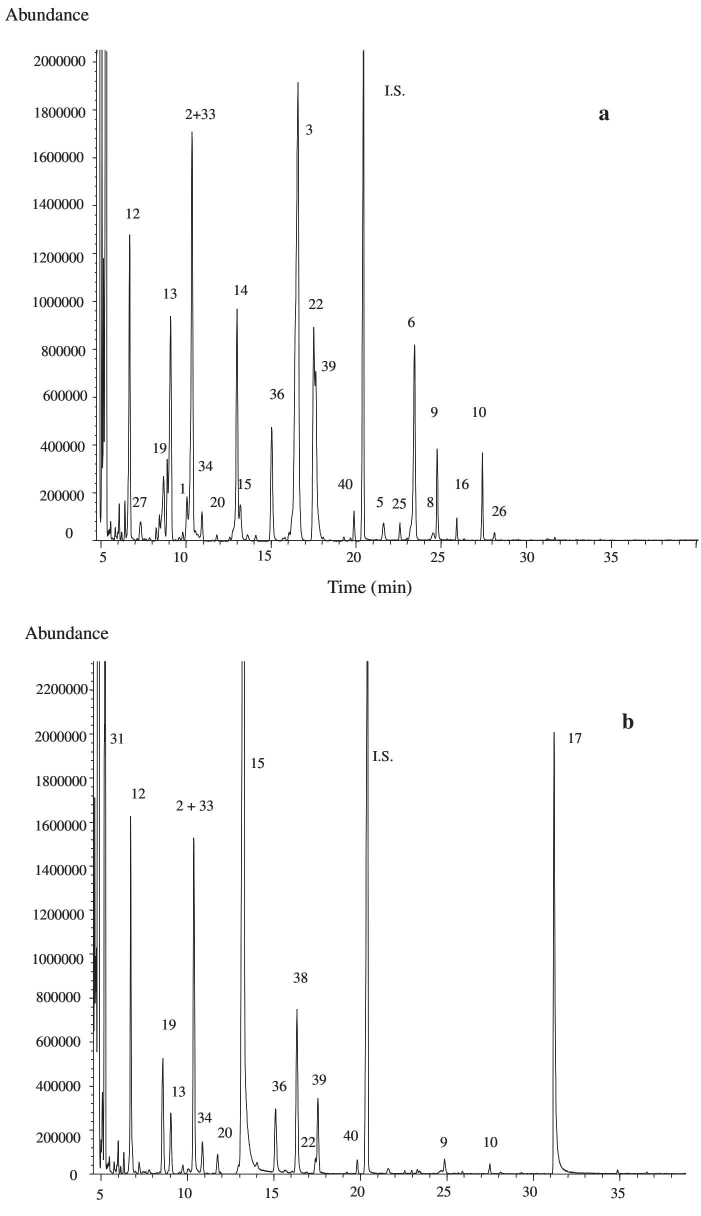

Figure 2. Different volatile compounds found by P&T at 12 months in artisanal cheese (a) and industrial cheese (b).

(a): propanone (12), 2-propenal (27), ethyl acetate (19), butanone (13), 2-propanol (1), ethanol + dichlorometane (2+33), benzene (34), ethyl propionate (20), 2-pentanone (14), diacetyl (15), acetonitrile (36), 2-butanol (3), ethyl butyrate (22), toluene (39), dimethyl disulfide (40), 2-hexanone (I.S.), 2-methyl-1-propanol (5), propyl butyrate (25), 2-pentanol (6), 1-methoxy-2-propanol (8), 1-butanol (9), 2-heptanone (16), 3 methyl-1-butanol (10), ethyl hexanoate (26);

(b): heptane (31), propanone (12), ethyl acetate (19), butanone (13), etanol + dichlorometane (2 + 33), benzene (34), ethyl propionate (20), diacetyl (15), acetonitrile (36), chloroform (38), ethyl butyrate (22), toluene (39), dimethyl disulfide (40), 2-hexanone (I.S.), 1-butanol (9), 3-methyl-1-butanol (10), acetoin (17).

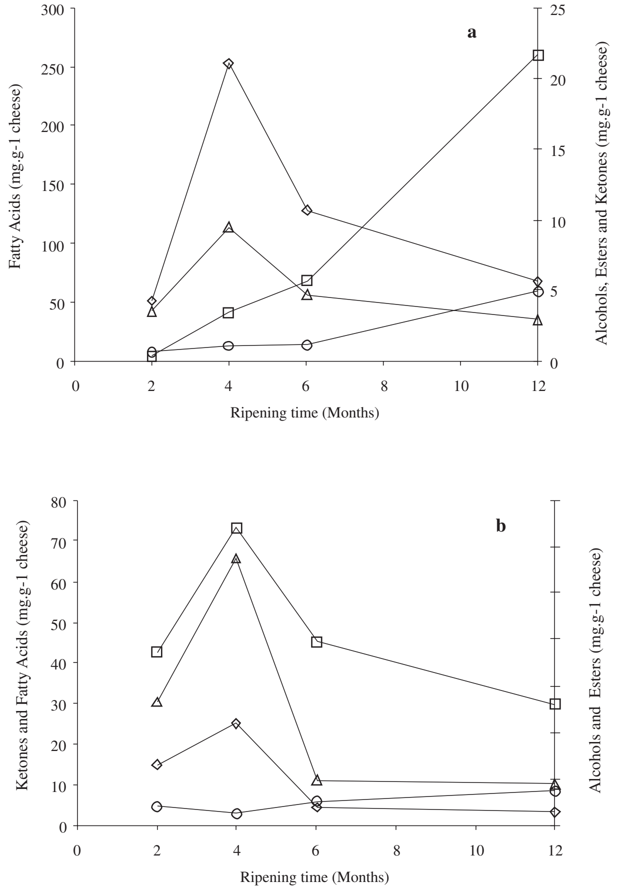

Figure 3. Evolution of the main groups of volatile compounds appearing in artisanal cheese (a) and in industrial cheese (b) by SDE: △ Alcohols, □ Fatty acids, ○ Esters, ◇ Ketones.

[35] and Cheddar [28]. These compounds can later be reduced to 2,3-butanediol, butanone and 2-butanol by adventitious bacteria [41].

The two analytical methods revealed different alcohols, especially in the artisanal cheeses. Most alcohols are formed from the corresponding aldehydes [8]. The most abundant primary alcohol was 3-methyl-l-butanol, which is presumed to originate from leucine. The main secondary alcohols were 2-heptanol and 2-nonanol, formed by reduction of methylketones [7]. The main alcohol in the P & T results for the artisanal cheeses was 2-butanol, present at higher levels than in industrial cheese samples. SDE disclosed a small amount of 2-phenylethanol in the industrial cheeses. Alcohols and esters are abundant in soft and mold-ripened cheeses [31] but even carbon number ethyl esters are present in most varieties of hard and semi-hard cheeses such as Los Beyos [12], Grana Padano [29] and Parmesan [4].

Different families of esters were detected, especially in the artisanal cheeses. The presence of the esters is probably related to esterase activity by lactic acid bacteria [29]. The main family comprised ethyl esters from acetate to hexadecanoate.

Table II. Volatile compounds found in Manchego cheeses by SDE.

Peak number	Compound	t_R	Cheese type		Identification	
			Artisanal	Industrial	GC	GC-MS
	Alcohols					
1	2-butanol	8.96	+		+	+
2	2-pentanol	11.11	+	+	+	+
3	3-methyl-2-butanol	13.01	+			+
4	3-methyl-1-butanol	13.72	+	+	+	+
5	2-hexanol	13.98		+		+
6	2-heptanol	17.35	+	+	+	+
7	1-hexanol	18.58	+		+	+
8	2-octanol	20.55	+		+	+
9	2-ethylhexanol	23.44			+	+
10	2-nonanol	24.31	+	+	+	+
11	1-octanol	25.69	+		+	+
12	2-undecanol	31.03	+	+	+	+
13	Benzenemethanol	36.99		+	+	+
14	2- phenylethanol	38.42		+		+
	Ketones					
15	Butanone	7.55	+			+
16	2-pentanone	8.72	+	+	+	+
17	2-heptanone	13.35	+	+	+	+
18	2-octanone	16.68	+	+	+	+
19	2-nonanone	20.28	+	+	+	+
20	Nonen-2-one	22.72	+	+		+
21	2-decanone	22.43	+	+	+	+
22	2-undecanone	27.5	+	+	+	+
23	2-tridecanone	34.41	+	+	+	+
24	2-pentadecanone	42.93	+	+	+	+
	Esters					
25	Ethyl propionate	7.93	+	+		+
26	Methyl butyrate	9.1	+			+
27	Ethyl butyrate	9.41	+	+	+	+
28	Butyl acetate	10.23	+			+
29	Propyl butyrate	11.51	+			+
30	Methyl hexanoate	13.46	+	+		+
31	Butyl butyrate	14.34	+			+
32	Ethyl hexanoate	14.82	+	+	+	+
33	Pentyl butyrate	15.9	+	+		+
34	Propyl hexanoate	17.69	+			+
35	Methylpropyl hexanoate	17.69	+			+
36	Ethyl heptanoate	18.2	+			+
37	Methyl octanoate	20.5		+		+

Table II. *(continued)*.

38	Butyl hexanoate	20.99	+			+
39	Ethyl octanoate	21.72	+	+	+	+
40	Methylbutyl hexanoate	22.59	+			+
41	Methyl decanoate	27.28	+	+		+
42	Ethyl decanoate	28.66	+	+	+	+
43	Ethyl dodecanoate	35.4	+		+	+
44	Ethyl tetradecanoate	44.2	+		+	+
45	Ethyl hexadecanoate	53.97	+		+	+
	Aldehydes					
46	Benzaldehyde	25.56	+	+	+	+
47	Phenylacetaldehyde	29.46	+	+		+
	Free fatty acids					
48	Butyric acid	28.66	+	+	+	+
49	3-methylbutyric acid	29.86	+			+
50	Hexanoic acid	35.43	+	+	+	+
51	Heptanoic acid	39.61	+	+	+	+
52	Octanoic acid	44.73	+	+	+	+
53	Nonanoic acid	51.28	+	+	+	+
54	Decanoic acid	60.62	+	+	+	+
55	Undecanoic acid	70.6	+	+	+	+
56	Dodecanoic acid	89.9	+	+	+	+
	Lactones					
57	δ-Decalactone	53.43		+		+
58	γ-Lactone	70.66		+		+
59	δ-Lactone	77.13		+		+
	Miscellaneous					
60	Methylcyclohexane	6.44	+	+		+
61	Dimethylcyclohexane	6.75		+		+
62	Benzene	7.87	+	+		+
63	Trichloroethane	8.66	+	+		+
64	Chloroform	9.44	+			+
65	Toluene	9.76	+	+		+
66	Alkylbenzene (C8H10)	12.38	+	+		+
67	Alkylbenzene (C8H10)	12.65	+	+		+
68	Alkylbenzene (C8H10)	12.81	+	+		+
69	Alkylbenzene (C9H12)	16.12	+	+		+
70	Alkylbenzene (C9H12)	16.66	+	+		+
71	Alkylbenzene (C9H12)	17.43	+	+		+
72	Alkylbenzene (C10H14)	23.48	+	+		+
73	Alkylbenzene C10H14)	27.50	+	+		+
74	C14H32	19.97		+		+
75	Limonene	14.8		+		+
76	2-butoxyethanol	20.67		+	+	+
77	Butylglycol acetate	22.38		+		+
78	2-butoxyethanol acetate	35.39		+		+

Table III. Volatile compounds found in Manchego cheeses by P&T.

Peak number	Compounds	t_R	Cheese type		Identification	
			Artisanal	Industrial	GC	GC-MS
	Alcohols					
1	2-propanol	10.07	+	+		+
2	Ethanol	10.33	+	+		+
3	2-butanol	16.77	+		+	+
4	1-propanol	17.87	+			+
5	2-methyl-1-propanol	21.62	+	+		+
6	2-pentanol	23.48	+		+	+
7	2,3-butanediol	24.64		+		+
8	1-metoxy-2-propanol	24.74	+			+
9	1-butanol	24.91	+	+		+
10	3-methyl-1-butanol	27.53	+	+	+	+
11	2-heptanol	31.73	+		+	+
	Ketones					
12	Propanone	6.68	+	+		+
13	Butanone	9.01	+	+		+
14	2-pentanone	12.9	+	+	+	+
15	Diacetyl	13.12	+	+		+
16	2-heptanone	25.9	+	+	+	+
17	Acetoin	31.32	+	+		+
18	2-nonanone	34.31	+	+	+	+
	Esters					
19	Ethyl acetate	8.54	+	+		+
20	Ethyl propionate	11.7	+	+		+
21	2-methylethyl propionate	12.16	+			+
22	Ethyl butyrate	17.48	+	+	+	+
23	2-methylethyl butyrate	18.51	+			+
24	Butyl acetate	19.83	+	+		+
25	Propyl butyrate	22.59	+			+
26	Ethyl hexanoate	28.08	+		+	+
	Aldehydes					
27	2-propenal	7.19	+			+
28	2-methylbutanal	9.66	+	+		+
29	3-methylbutanal	9.69	+	+		+

Table III. *(continued)*.

	Miscellaneous					
30	Cyclopentane	4.85		+		+
31	Heptane	5.24	+	+		+
32	Octane	6.27	+	+		+
33	Dichloromethane	10.33	+	+		+
34	Benzene	10.86	+	+	+	+
35	Trichloroethane	14.02	+	+		+
36	Acetonitrile	15.1	+	+		+
37	Tetrachlorethane	16.04		+		+
38	Chloroform	16.32	+	+		+
39	Toluene	17.54	+	+	+	+
40	Dimethyl disulfide	19.81	+	+		+
41	Ethylbenzene	22.57	+	+		+
42	Dimethyl benzene	22.97		+		+
43	2-butoxyethanol	34.88		+	+	+
44	Butyl glycol acetate	36.57		+	+	+

Some methyl, propyl, butyl, pentyl and branched esters were also present.

Aldehydes are produced from amino acids either by transamination followed by decarboxylation or by Strecker degradation [41]; they are easily reduced to alcohols [31]. Thus, 2-methylbutanal and 3-methylbutanal could be produced from isoleucine and leucine, respectively, benzaldehyde and phenylacetaldehyde from phenylalanine, and 2-propenal could originate from methional in methionine metabolism [45].

Several chlorinated compounds that might be from endogenous (halocarbons from drinking water, cleaning agents, or pesticides) or exogenous (from the laboratory atmosphere) origin were detected. The presence of aromatic compounds such as alkyl benzenes, previously reported in other cheeses, has not been satisfactorily explained, although some authors have postulated that they might be breakdown products of carotene in milk [20].

Substances such as 2-butoxyethanol, butyl glycol acetate and 2-(2-butoxyethoxy) ethanol acetate were present only in the industrial cheeses, being more abundant in the rind. These compounds have not been described as normal metabolites of cheese microorganisms; on the contrary, some of them have been reported in foods as arising from package [32]. These substances are used as additives for paints, lacquers, coatings and others. This may explain their presence in the industrial cheese rind.

3.3. Connection between odour characteristics and volatile composition

Odour intensity was higher in the artisanal cheeses, but it is difficult to establish a relation between this attribute and the volatile concentrations. Thus, it would seem to be more appropriate to attribute some of the odour notes perceived by the panellists to the main volatiles found.

FFAs are probably the principal volatile components present in Manchego cheeses [5, 37, 38]. The pungent sensation detected in the artisanal cheeses could also be ascribable to the high FFA content in the samples, whereas butyric acid and the medium chain

FFAs (hexanoic, octanoic and decanoic acids) could be responsible for the rancid overtones. Neither pungent nor rancid notes were perceived in the industrial cheeses, probably because of their lower FFA content and perhaps the effect of other components.

Methylketones are present in most hard cheeses, but at lower levels than in mold-ripened cheeses [31], in which they are major aroma components. They were present at similar levels in both types of cheeses here, and they probably contribute to background aroma, without stand-out notes. The fruity or floral odours described for some methylketones, e.g. 2-nonanone [40] could help to explain the fruity overtones detected in the industrial cheeses.

The butter odour perceived in the industrial cheeses could be attributed to diacetyl, which was one of the main peaks found in these samples by P&T. Acetoin may also contribute, although its odour threshold is higher. Both compounds were present at lower concentrations in the artisanal cheeses, in which no butter odour was perceived.

It is difficult to assign the lactic/yoghurt odour to a specific group of compounds. The main volatile compounds reported to be responsible for yoghurt odour are acetaldehyde, diacetyl and acetone [21] and also acetoin and butanone [23]. These compounds, minus acetaldehyde, were present in the industrial cheeses, although in proportions different from those reported for yoghurt. Lactic acid is not usually regarded as a volatile compound, but it was present in the cheeses and does have a slightly perceptible fresh odour which could contribute to the lactic/yoghurt note reported by the taste panellists.

Esters afford different fruity odours, and the presence of many different esters in the cheeses was probably the source of this mild, pleasant note. It should be noted that the 12-month-old industrial cheeses with lower amounts of esters than the artisanal cheeses had fruity overtones, whereas the artisanal cheeses did not; this odour was probably masked in these cheeses by the more intense odours in relation to the higher FFA levels.

Lactones are relevant components of flavour in some cheeses and they seem to be related to the use of pasteurised milk in cheesemaking [29, 40]. Traces of some lactones were found in the industrial samples (made with pasteurised milk) but it is difficult to say if they play some role in the fruity notes of these samples.

Hay odours in dairy products have been attributed to certain aldehydes and unsaturated alcohols which were not detected in our samples. Perhaps other aldehydes and alcohols could account for this descriptor as a result of different "green", "herbaceous" or floral odours (for example, phenylacetaldehyde or 2-phenylethanol, which have a floral, rose-like odour), and terpenes such as limonene, which were present at very low concentrations.

Other odour descriptors such as milky odour and briny odour are difficult to relate to specific compounds. Milk in this case means ovine milk, which has a very complex aroma, insufficiently defined [30]. This odour was less pronounced (and ultimately undetectable) in the artisanal cheeses, probably due to the effect of the FFAs, whereas it was more readily perceivable in the industrial cheeses, which had a milder flavour.

Compounds such as dimethyl disulfide, nonen-2-one, and others with very low odour thresholds may also have contributed to the overall odour.

Although it is difficult to establish a quantitative relationship between cheese composition and the sensory effect of these compounds, the work described above allowed us to qualitatively relate most of the sensory notes of the studied cheeses to the main groups of volatile components present.

Further experiments will be necessary in order to improve our knowledge of the Manchego aroma.

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