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Volatile fraction and sensory characteristics of Manchego cheese. 1. Comparison of raw and pasteurized milk cheese

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Summary. Manchego cheese can be manufactured from raw or pasteurized ewes' milk. An automatic purge and trap apparatus, coupled to a GC-MS was used to isolate, identify and compare the relative amounts of the volatile components of raw and pasteurized Manchego cheese during ripening. The majority of volatile compounds were more abundant in raw milk (RM) cheeses than in pasteurized milk (PM) cheeses. Alcohols and esters predominated in the profile of RM Manchego cheese, while methyl-ketones and 2,3-butanedione were quantitatively important in PM cheeses. Branched chain alcohols were much more abundant in RM cheeses. The discriminant analysis separated 100 % samples into RM or PM cheeses by using only 16 volatile compounds. Aroma intensity was correlated with esters, branched chain aldehydes and branched chain alcohols in RM cheeses, and with esters, branched chain aldehydes, 2-methyl ketones and 2-alkanols in PM cheeses. Diacetyl was positively correlated with the aroma attribute 'toasted' and negatively correlated with aroma quality in PM cheeses.

Keywords: Volatile compounds, raw milk, pasteurized milk, Manchego cheese ripening.

The chemical and enzymatic reactions leading to the transformation of curd into mature cheese are highly complex. During cheese ripening hundreds of chemical compounds accumulate which contribute in different ways to the cheese flavour (Fox & Wallace, 1997; McSweeney & Sousa, 2000). Many factors influence the final aroma of a particular variety of cheese, including milk origin, milk treatment, type and amount of starter added, manufacture conditions and ripening time and temperature. Pasteurization of the milk and starter addition results in a more hygienic product and provides a more homogeneous quality to the cheese. For these reasons most cheeses in the world are nowadays produced from pasteurized milk (PM). However, raw milk (RM) cheeses represent a significant proportion of the ripened cheeses produced in Europe (Grappin & Beuvier, 1997). PM cheeses usually mature at a slower rate and lack the sensory peculiarities of RM cheeses (Grappin & Beuvier, 1997). In RM cheeses, milk native enzymes and wild microbiota are acting, not

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always beneficially, as the main producers of flavour compounds. McSweeney *et al.* (1993) and Beuvier *et al.* (1997) studied Cheddar and Swiss-type cheeses made from raw, pasteurized and microfiltered milk, finding considerable differences in the microbiota, free amino acids, free fatty acids and sensory characteristics, although no other volatile compounds than free fatty acids (FFA) were determined. Buchin *et al.* (1998) found significant differences in the volatile fractions of experimental semi-hard cheeses from PM and RM. Chavarri *et al.* (2000) reported lower levels of FFA in cheeses made from ewes' PM, as compared with RM cheeses.

Manchego cheese is the best known among the Spanish ewes' milk cheeses possessing a Protected Designation of Origin (PDO). It is a semi-hard or hard cheese variety made in the La Mancha region from raw or pasteurized ewes' milk obtained from the Manchega breed, using bovine rennet as coagulant, and usually adding mesophilic starter cultures. Manchego cheeses are cylindrical in shape and weigh 2–3 kg each. The time Manchego cheese is consumed varies widely, but usually RM cheeses are commercialized after 3–6 months ripening, and PM cheeses with 6–9 months ripening. Traditional Manchego cheese from RM possesses an aroma characteristic of sheep milk, slightly lipolytic and pungent, whereas the Manchego cheese made from PM has a milder flavour with lactic notes. Several Spanish research groups have reported on microbiological and physicochemical characteristics of Manchego cheese (Marcos, 1987; Nuñez *et al.* 1989), but studies of the volatile fraction are scarce. Gaya *et al.* (1990) reported lower lipolysis in Manchego cheeses made from PM than in those from RM. Martínez-Castro *et al.* (1991) found FFA, methyl ketones and methyl and ethyl esters in the medium volatility fraction of Manchego cheese from pasteurized milk by using the simultaneous distillation extraction technique (SDE). Villaseñor *et al.* (2000) found a high variability in the headspace of commercial samples of Manchego cheeses from different origins, especially between those from PM or RM.

The actual contribution of each volatile compound to the flavour of cheeses remains largely unknown, but the comparative study of PM and RM Manchego cheese could indirectly help to understand which compounds are imparting specific flavours or are related to aroma intensity or quality.

The objective of this study was to isolate, identify and compare the relative amounts of the volatile components present at different stages of ripening in RM and PM PDO Manchego cheeses made throughout the year. The correlations of certain volatile compounds with sensory characteristics of cheeses are also discussed.

Materials and Methods

Cheeses

The cheeses for this study were part of the normal production of five dairies, all located within an area of 110 km diameter in La Mancha region. Three artisan dairy farms transforming RM and two large industries transforming PM were selected among the PDO Manchego cheese manufacturers. Vats of 1000–2000 kg were used in small dairies, adding to RM DVI mesophilic N-type starters. Vats of 10000–25000 kg were used in the large industries, adding to the PM (73 °C for 16 s), a mixture of N-type and LD-type bulk starter cultures. Bovine rennet was used to coagulate milk in 30–40 min at 31–32 °C. Curd was cut to rice grain size and scalded at 36–37 °C for 30 min. Cheeses were pressed for 3–6 h, and brine salted for 24–36 h. Yield after pressing was slightly above 1 kg cheese per 5 kg milk. RM cheeses ripened at

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11–12 °C and 80–90 % RH during 2 months, and then were held at 6 °C. PM cheeses dried off at 12–14 °C and 70 % RH during 12 d and ripened at 6–8 °C and 85 % RH afterwards.

Duplicate batches, 2 weeks apart, were made per dairy in each season (i.e. total of eight batches per dairy). RM cheeses were analysed at 3 and 6 months of ripening and PM cheeses at 3, 6 and 9 months of ripening. A different cheese was analysed at each sampling time. Therefore, 48 RM cheeses and 48 PM cheeses were analysed for volatile compounds and sensory characteristics. For the studies of volatile compounds, cheeses were cut in sectors, the rind was removed, and the pieces were wrapped in aluminium foil, vacuum packed and stored at −40 °C until GC analysis. Cheese sectors were thawed at 5 °C overnight, and then kept at room temperature for 2 h before GC analysis. Analyses were carried out in duplicate.

Purge and trap procedure and GC-MS

For the extraction and injection of the volatile components, an automatic purge and trap apparatus (HP 7695, Hewlett-Packard, Palo Alto, CA, USA) was used, coupled to a gas chromatograph–mass spectrometer (HP-6890). Optimization of the sample preparation and purge conditions was necessary. After assaying different purge temperatures, purge times, He flows, trap pressures, trap temperatures, and different amounts of Na_2SO_4 added to obtain a salting-out effect, the final compromise procedure was as follows: A 15-g triangular piece of cheese was cut from the centre of the cheese sector, parallel to the circular surface, and homogenized in an analytical grinder (IKA Labortechnik, Staufen, Germany) with 20 g Na_2SO_4 and 75 μl internal standard aqueous solution prepared with 0·5 mg cyclohexanone and camphor/ml (Sigma-Aldrich Química, Alcobendas, Spain). The mixture (2 g) was subjected to dynamic headspace at 50 °C, during 15 min using He (45 ml/min), with 10 min previous equilibration. Volatile compounds were concentrated in a Tenax trap (Tekmar, Cincinnati, OH, USA) maintained at 25 °C and 6·5 psi backpressure. The transfer line, moisture trap and valves were kept at 200 °C. The trap was subjected to dry purge for 0·5 min, and volatile compounds were desorbed during 0·5 min at 230 °C directly into the injection port at 220 °C, with a split ratio of 1:20, and 1·4 ml He/min flow.

Gas chromatography was carried out in a GC-MS (HP-6890) apparatus equipped with a capillary column HP-Innowax (60 m × 0·25 mm o.d., film thickness 0·5 μm, Agilent Technologies, Las Rozas, Spain). Chromatographic conditions were as follows: 17 min at 45 °C, 4 deg C/min to 110 °C, 10 min at 110 °C, 15 deg C/min to 240 °C, He flow: 1 ml/min. Total analysis time was 55 min. Detection was performed with the mass selective detector (HP 5973) operating in the scan mode, 2·6 scan/s, with 70 eV IE, source and quadrupole temperatures of 230 °C and 150 °C, respectively. Peak identification was by comparison of retention times with real standards (Sigma-Aldrich Química), and comparison of spectra with bibliographic data from the Wiley 275 library (Wiley & Sons, Inc., Germany). Quantification was carried out by sum of all ions abundance (TIC), with reference to the cyclohexanone peak. The exceptions were 2-butanol and 1-propanol, which were quantified by ion 59, and ethyl butyrate, coeluting with 1-propanol, which was quantified by ion 88. Data are presented as relative abundance. A TI abundance of approximately 10^5 units, about three times the noise area, was considered the detection limit.

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Sensory analysis

Fourteen trained panellists tasted cheeses for quality and intensity of odour and aroma on a 0–7 points scale. A representative slice of a maximum of three cheeses per session was presented in closed Petri dishes. Odour was defined as the olfactory sensation felt directly by smelling the cheese; aroma was defined as the olfactory sensation felt in the retro nasal way upon mastication. A descriptive test was developed for Manchego cheese based on the guidelines to the smell and aroma evaluation of hard and semi-hard cheeses given by Berodier *et al*. (1997). Panellists were asked to give a score on a 0–3 points scale (0 = undetected; 1 = mild; 2 = medium; 3 = strong) to the following flavour attributes: families 'lactic', 'fruits-flowers', 'vegetal', 'animal', 'toasted', and individual attribute descriptors 'rancid', 'pungent', and 'sheepy'.

Statistics

Statistical analysis of the data was performed with the SPSS Win 5.0 program. Analysis of variance ($\alpha = 95\%$) was carried out using ripening time and type of milk as main effects. A stepwise discriminant analysis was performed to determine those volatile compounds most useful in classifying the samples into RM or PM cheeses, using the Wilk's lambda as the statistical selection criterion for the variables. In order to correlate the volatile compounds results with the sensory data, sums of abundance for chemical families, as presented in Tables 1–4, were calculated in order to make them more manageable. Sums of volatile compounds, along with certain individual volatile compounds, scores for aroma quality, aroma intensity, and descriptors, were subjected to a 2-factor principal component analysis (PCA) with Varimax rotation. Principal components analyses were carried out separately for RM and PM cheeses.

RESULTS

Chromatographic analysis of volatile compounds

The optimized procedure for purge and trap extraction coupled to GC-MS analysis allowed the correct separation of 77 peaks corresponding to 83 volatile compounds in the headspace of RM and PM Manchego cheeses during ripening. The coefficient of variation of the method ranged from approximately 10 to 20%, depending on the compound. The use of the internal standard method for quantification allows the comparison of the volatile fraction of cheeses possessing similar characteristics. Determination of absolute concentrations of the volatile compounds would have required the use of the standard addition method to avoid the matrix effect, and only to a certain extent.

Tables 1–4 show the relative abundance of the volatile compounds of RM and PM Manchego cheeses during ripening. Seven peaks belonged to unresolved species, and the results correspond to the sum. The variability of the abundances among the cheeses made at different seasons and in different dairies was considerable for both RM and PM cheeses, as shown by the high standard deviations of most compounds. This variability has been the subject of a detailed study (Fernández-García *et al*. 2002).

The relative abundance of carbonyl compounds (aldehydes and ketones) in the volatile fraction of Manchego cheeses at 3, 6 and 9 months ripening are shown in Table 1. Significantly higher concentrations ($P < 0{\cdot}05$) of propanal and 2-propenal were found in RM Manchego cheeses. Branched chain aldehydes increased signi-

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Table 1. *Relative abundance*[1] *of the carbonyl compounds detected in the volatile fraction of raw milk* (*RM*) *and pasteurized milk* (*PM*) *Manchego cheese during ripening*

(Values are means ± SD for $n = 16$ or 24)

Carbonyl compounds	3 months		6 months		9 months	Significance of ripening time[2]	
	RM cheese ($n = 24$)	PM cheese ($n = 16$)	RM cheese ($n = 24$)	PM cheese ($n = 16$)	PM cheese ($n = 16$)	RM	PM
Aldehydes	5·78 ± 6·22	3·81 ± 3·01	6·26 ± 6·23	5·61 ± 4·80	5·76 ± 4·33	NS	NS
Acetaldehyde	1·71 ± 1·24	1·68 ± 1·54	1·78 ± 0·85	2·24 ± 2·23	1·33 ± 0·41	NS	NS
n-Propanal	0·36 ± 0·42[a]	0·17 ± 0·06[b]	0·42 ± 0·33[a]	0·18 ± 0·08[b]	0·21 ± 0·20	NS	NS
2-Propenal	1·55 ± 2·62[a]	0·12 ± 0·28[b]	1·66 ± 2·15[a]	0·18 ± 0·32[b]	0·17 ± 0·34	NS	NS
n-Hexanal + 2-hexanone	1·41 ± 0·79	1·30 ± 0·52	1·62 ± 1·06[b]	2·39 ± 1·56[a]	2·26 ± 1·34	**	**
n-Nonanal	0·43 ± 0·68	0·27 ± 0·33	0·42 ± 0·47	0·34 ± 0·37	1·05 ± 1·21	NS	***
n-Decanal	0·32 ± 0·47	0·27 ± 0·33	0·36 ± 0·28	0·28 ± 0·24	0·74 ± 0·83	NS	**
Branched chain aldehydes	2·99 ± 1·91	2·47 ± 1·48	4·75 ± 2·41[b]	5·91 ± 3·97[a]	7·45 ± 3·68	***	***
2 Methyl-propanal	0·36 ± 0·34	0·41 ± 0·27	0·46 ± 0·36[b]	0·70 ± 0·60[a]	1·00 ± 0·69	NS	***
2-Methyl-1-butanal	0·33 ± 0·32[a]	0·17 ± 0·12[b]	0·65 ± 0·40	0·57 ± 0·37	0·79 ± 0·47	***	***
3-Methyl-1-butanal	2·30 ± 1·25	1·89 ± 1·09	3·64 ± 1·65	4·64 ± 3·00	5·66 ± 2·52	***	***
Methyl ketones (*sum except C3–C4*)	21·07 ± 26·59	23·53 ± 30·52	56·31 ± 66·37	79·41 ± 74·30	132 ± 107	**	***
2-Propanone	9·78 ± 13·53	17·19 ± 6·63	6·59 ± 5·61[b]	20·3 ± 10·6[a]	29·87 ± 12·97	NS	***
2-Butanone	175 ± 131[a]	9·11 ± 14·8[b]	172 ± 132[a]	21·0 ± 38·6[b]	70·05 ± 212	NS	NS
2-Pentanone	10·26 ± 11·12	18·0 ± 24·8	25·79 ± 21·62[b]	57·0 ± 45·2[a]	96·13 ± 65·12	***	***
2-Heptanone	9·22 ± 12·6	4·98 ± 5·25	23·86 ± 29·96	19·27 ± 23·51	31·0 ± 34·4	**	***
2-Octanone	0·16 ± 0·49	0·02 ± 0·05	0·32 ± 0·51	0·19 ± 0·23	0·25 ± 0·36	NS	**
2-Nonanone	1·34 ± 2·29	0·51 ± 0·39	6·26 ± 14·20	2·90 ± 5·31	4·79 ± 7·17	*	*
2-Undecanone	0·09 ± 0·09[a]	0·02 ± 0·03[b]	0·08 ± 0·08[a]	0·05 ± 0·05[b]	0·04 ± 0·05	NS	*
Diketones and reduction products	20·16 ± 24·14[b]	207 ± 148[a]	10·09 ± 7·64[b]	204 ± 167[a]	234 ± 155	*	NS
2,3-Butanedione	18·11 ± 20·41[b]	179 ± 116[a]	8·75 ± 5·72[b]	176 ± 131[a]	210 ± 132	**	NS
2,3-Pentanodione	0·17 ± 0·32[b]	1·21 ± 0·86[a]	0·13 ± 0·18[b]	1·05 ± 0·68[a]	1·53 ± 1·45	NS	NS
2,3-Heptanodione	0·83 ± 2·20	0·03 ± 0·06	0·34 ± 0·49[a]	0·09 ± 0·22[b]	0·05 ± 0·07	NS	NS
3-Hydroxy-2-butanone	1·02 ± 1·1[b]	26·2 ± 30·6[a]	0·83 ± 1·16[b]	27·1 ± 34·6[a]	22·1 ± 21·2	NS	NS
3-Hydroxy-2-pentanone	0·03 ± 0·11[b]	0·24 ± 0·36[a]	0·04 ± 0·09[b]	0·21 ± 0·28[a]	0·17 ± 0·32	NS	NS

[1] Relative abundance expressed as percentage of the cyclohexanone peak. [2] Significance of ripening time: NS, $P > 0·05$; * $P \leqslant 0·05$; ** $P \leqslant 0·01$; *** $P \leqslant 0·001$. [ab] Means followed by a different letter within the same ripening time were significantly different ($P < 0·05$).

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Table 2. *Relative abundance*[1] *of alcohols detected in the volatile fraction of raw milk (RM) and pasteurized milk (PM) Manchego cheese during ripening*

(Values are means ± SD for $n = 16$ or 24)

Alcohols	3 months RM cheese ($n = 24$)	3 months PM cheese ($n = 16$)	6 months RM cheese ($n = 24$)	6 months PM cheese ($n = 16$)	9 months PM cheese ($n = 16$)	Significance of ripening time[2] RM	Significance of ripening time[2] PM
Primary alcohols (except ethanol)	74·11 ± 83·75[a]	15·27 ± 14·57[b]	112 ± 113[a]	15·19 ± 16·24[b]	25·18 ± 41·27	*	NS
Ethanol	756 ± 404[a]	243 ± 141[b]	680 ± 295[a]	253 ± 111[b]	244 ± 131	NS	NS
1-Propanol	35·8 ± 36·2[a]	0·50 ± 0·36[b]	48·6 ± 42·0[a]	0·61 ± 0·26[b]	6·59 ± 21·54	NS	NS
2-Propen-1-ol	12·41 ± 17·77[a]	ND[b]	9·13 ± 11·15[a]	ND[b]	0·06 ± 0·16	NS	NS
1-Butanol	14·98 ± 18·86[a]	1·16 ± 0·44[b]	38·21 ± 40·92[a]	2·43 ± 2·21[b]	3·49 ± 2·58	***	***
1-Pentanol + 3-methyl-3-buten-1-ol	2·04 ± 0·54	2·18 ± 0·49	1·87 ± 0·48[b]	2·19 ± 0·90[a]	1·94 ± 1·01	NS	NS
1-Hexanol	1·27 ± 1·11[a]	0·27 ± 0·08[b]	4·65 ± 5·67[a]	0·33 ± 0·07[b]	0·40 ± 0·29	***	*
2-Butoxy-ethanol	7·30 ± 9·12	10·93 ± 13·11	8·78 ± 13·08	9·34 ± 12·7	12·37 ± 15·51	NS	NS
1-Heptanol	0·14 ± 0·08	0·13 ± 0·05	0·16 ± 0·13	0·17 ± 0·07	0·19 ± 0·14	NS	*
1-Octanol	0·17 ± 0·07[a]	0·10 ± 0·04[b]	0·27 ± 0·19[a]	0·12 ± 0·03[b]	0·14 ± 0·04	**	***
Secondary alcohols (except C3–C4)	38·00 ± 47·84[a]	2·74 ± 2·99[b]	96·4 ± 105[a]	19·53 ± 22·20[b]	27·84 ± 38·39	***	**
2-Propanol	132 ± 129[a]	22·42 ± 30·50[b]	163 ± 137[a]	19·9 ± 17·3[b]	29·5 ± 30·0	NS	NS
2-Butanol	295 ± 222[a]	0·42 ± 0·41[b]	366 ± 226[a]	1·35 ± 3·04[b]	26·43 ± 87·7	NS	NS
2-Pentanol	34·0 ± 41·9[a]	2·32 ± 3·52[b]	86·1 ± 89·2[a]	18·16 ± 20·4[b]	24·94 ± 32·5	***	**
2-Hexanol	0·31 ± 0·46[a]	0·03 ± 0·06[b]	1·20 ± 2·10[a]	0·15 ± 0·18[b]	0·23 ± 0·29	**	**
2-Heptanol	3·42 ± 5·11[a]	0·35 ± 0·55[b]	8·75 ± 13·3[a]	1·16 ± 1·50[b]	2·49 ± 5·22	*	*
2-Nonanol	0·13 ± 0·14[a]	0·01 ± 0·03[b]	0·27 ± 0·27[a]	0·05 ± 0·10[b]	0·14 ± 0·33	**	*
2-Undecanol	0·11 ± 0·23	0·03 ± 0·06	0·08 ± 0·08[a]	0·01 ± 0·02[b]	0·04 ± 0·05	NS	NS
Branched chain alcohols	156 ± 204[a]	6·89 ± 3·53[b]	147 ± 182[a]	11·7 ± 7·84[b]	14·21 ± 12·04	NS	**
2-Methyl-1-propanol	35·4 ± 49·9[a]	2·73 ± 1·21[b]	33·6 ± 43·8[a]	3·90 ± 1·60[b]	4·69 ± 2·52	NS	***
3-Methyl-1-butanol	121 ± 160[a]	4·16 ± 2·32[b]	113 ± 138[a]	7·80 ± 6·24[b]	9·52 ± 9·52	NS	*

[1] Relative abundance expressed as percentage of the cyclohexanone peak. [2] Significance of ripening time: NS, $P > 0·05$; * $P \leqslant 0·05$; ** $P \leqslant 0·01$; *** $P \leqslant 0·001$. ND, below detection limit. [ab] Means followed by a different letter within the same ripening time were significantly different ($P < 0·05$).

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Table 3. *Relative abundance*[1] *of esters detected in the volatile fraction of raw milk (RM) and pasteurized milk (PM) Manchego cheese during ripening*

(Values are means ± SD for $n = 16$ or 24)

Esters	3 months		6 months		9 months	Significance of ripening time[2]	
	RM cheese ($n = 24$)	PM cheese ($n = 16$)	RM cheese ($n = 24$)	PM cheese ($n = 16$)	PM cheese ($n = 16$)	RM	PM
Methyl esters	2·10 ± 3·60[a]	0·68 ± 0·51[b]	5·24 ± 5·12[a]	1·12 ± 0·96[b]	1·67 ± 2·99	**	*
Methyl acetate	0·35 ± 0·38	0·29 ± 0·28	0·41 ± 0·27	0·47 ± 0·47	0·82 ± 2·18	NS	NS
Methyl butyrate + 2-butyl acetate	1·34 ± 2·98[a]	0·024 ± 0·058[b]	4·08 ± 4·33[a]	0·18 ± 0·33[b]	0·20 ± 0·38	***	*
Methyl hexanoate + n-heptanal	0·41 ± 0·24	0·37 ± 0·17	0·75 ± 0·52[a]	0·47 ± 0·16[b]	0·65 ± 0·43	*	**
Ethyl esters	16·09 ± 14·81[a]	1·15 ± 1·02[b]	31·34 ± 26·37[a]	3·57 ± 3·96[b]	5·95 ± 6·06	***	**
Ethyl acetate	6·75 ± 6·69[a]	0·72 ± 0·36[b]	10·55 ± 11·05[a]	2·54 ± 3·12[b]	3·61 ± 3·87	**	**
Ethyl butyrate	2·32 ± 1·83[a]	0·17 ± 0·33[b]	5·21 ± 3·84[a]	0·38 ± 0·31[b]	0·92 ± 0·82	***	***
Ethyl pentanoate	0·08 ± 0·11[a]	ND[b]	0·23 ± 0·20[a]	ND[b]	0·02 ± 0·04	***	*
Ethyl hexanoate	6·31 ± 5·59[a]	0·22 ± 0·27[b]	13·9 ± 10·6[a]	0·55 ± 0·45[b]	1·24 ± 1·15	***	***
Ethyl octanoate	0·63 ± 0·59[a]	0·04 ± 0·06[b]	1·45 ± 1·12[a]	0·10 ± 0·08	0·16 ± 0·18	***	***
Higher esters (sum except butyl glycol ac.)	8·22 ± 14·53[a]	1·48 ± 1·01[b]	15·93 ± 25·12[a]	2·30 ± 2·36[b]	2·17 ± 2·53	***	NS
Propyl acetate	1·29 ± 2·41[a]	ND[b]	5·60 ± 7·90[a]	ND[b]	0·42 ± 1·57	***	*
Butyl acetate	1·25 ± 3·98	0·18 ± 0·14	2·85 ± 3·52[a]	0·61 ± 0·63[b]	0·31 ± 0·14	*	**
2-Propenyl butyrate	0·05 ± 0·13	ND	0·079 ± 0·13[a]	ND[b]	ND	NS	NS
Butyl butyrate	0·04 ± 0·09[a]	ND[b]	0·29 ± 0·45[a]	0·013 ± 0·05[b]	0·01 ± 0·03	***	NS
Propyl hexanoate + 3-methyl-2-buten-1-ol	1·03 ± 0·51[a]	0·64 ± 0·27[b]	1·85 ± 1·53[a]	0·62 ± 0·33[b]	0·68 ± 0·30	***	NS
Butyl hexanoate	0·006 ± 0·03	ND	0·07 ± 0·15[a]	ND[b]	ND	**	NS
Butyl glycol acetate	4·55 ± 7·38[a]	0·66 ± 0·60[b]	5·19 ± 8·44[a]	1·06 ± 1·35[b]	0·75 ± 0·49	NS	NS
Branched chain alkyl esters	6·61 ± 4·86[a]	0·13 ± 0·08[b]	19·76 ± 21·64[a]	0·36 ± 0·36[b]	0·76 ± 1·39	***	*
2-Methyl propyl acetate	0·17 ± 0·30[a]	ND[b]	0·61 ± 0·97[a]	0·005 ± 0·03[b]	0·06 ± 0·15	**	*
3-Methyl-1-butyl acetate + propyl butyrate	3·42 ± 3·20[a]	ND[b]	10·94 ± 9·24[a]	0·03 ± 0·06[b]	0·24 ± 0·70	***	NS
2-Methyl propyl butyrate + ethyl benzene	2·31 ± 0·34[a]	0·10 ± 0·02[b]	5·68 ± 7·97[a]	0·23 ± 0·15[b]	0·22 ± 0·14	***	NS
3-Methyl-1-butyl butyrate	0·44 ± 0·58[a]	0·03 ± 0·06[b]	0·98 ± 1·11[a]	0·09 ± 0·11[b]	0·21 ± 0·36	**	**
2-Methyl-propyl hexanoate	0·24 ± 0·39[a]	ND	1·49 ± 2·27[a]	ND[b]	ND	***	NS
2-Methyl-1-propyl octanoate	0·03 ± 0·05[a]	ND[b]	0·06 ± 0·08[a]	0·003 ± 0·01[b]	0·03 ± 0·04	*	***

[1] Relative abundance expressed as percentage of the cyclohexanone peak. [2] Significance of ripening time: NS, $P > 0·05$; * $P \leqslant 0·05$; ** $P \leqslant 0·01$; *** $P \leqslant 0·001$. [3] ND, below detection limit. [ab] Means followed by a different letter within the same ripening time were significantly different ($P < 0·05$).

ficantly ($P < 0{\cdot}001$) during ripening in all cheeses. At 3 months ripening branched chain aldehydes showed, in general, lower concentrations in PM cheeses, but at 6 months ripening 2-methyl propanal and 3-methyl butanal showed higher concentrations in PM than in RM cheeses. Major ketones were 2-propanone, 2-butanone, 2-pentanone and 2-heptanone. Significantly higher quantities ($P < 0{\cdot}05$) of 2-butanone were found in RM cheeses. 2-Pentanone, 2,3-butanedione (diacetyl) and 2,3-pentanedione abundances were significantly higher ($P < 0{\cdot}05$) in PM cheeses. Most methyl ketones increased significantly with ripening time ($P < 0{\cdot}05$), especially in PM cheeses, while 2,3 butanedione abundance decreased with ripening time in RM cheeses.

Alcohols were quantitatively the main chemical family found in the volatile fraction of Manchego cheese (Table 2). Large quantities of ethanol, 2-propanol, 2-butanol and 3-methyl-1-butanol (together with 2-methyl-1-butanol which coelutes with it), were observed. In general n-alkanols were significantly ($P < 0{\cdot}05$) more abundant in RM cheeses, especially ethanol, 1-propanol, 2-propen-1-ol, 1-butanol and 1-hexanol. All 2-alkanols and branched chain alcohols had significantly ($P < 0{\cdot}001$) higher concentrations in RM cheeses. The presence of 2-butoxy-ethanol depended on the dairy. Butanol, hexanol, octanol and most 2-alkanols increased with ripening time in both RM and PM cheeses. Branched chain alcohols increased significantly during ripening only in PM cheeses.

Twenty-three species of esters were identified in the volatile fraction of Manchego cheese (Table 3). Some esters coeluted and are given as sums. Ethyl, propyl, branched chain alkyl and butyl glycol esters were quantitatively important. At 6 months ripening, all esters, except methyl acetate, were significantly more abundant in RM cheeses, levels being 2- to 20-fold those found in PM cheeses. Some esters had very low concentrations or were not found in PM cheeses, even at 9 months ripening, when only ethyl esters of acetic, butyric and hexanoic acids were relatively abundant. Esters increased during ripening in both RM and PM cheeses (Table 3).

Table 4 summarizes the abundances of hydrocarbons, terpenes, FFA, and sulphur, aromatic and nitrogen compounds found in Manchego cheese during ripening. 1,3-pentanediene, 3-methyl-1-heptene and α-pinene were significantly more abundant, and limonene and p-cymene less abundant, in PM cheeses. In general no significant differences ($P > 0{\cdot}05$) were found between the aromatic compounds or FFA content of RM and PM cheeses. Dimethyl sulphide and dimethyl disulphide were significantly ($P < 0{\cdot}05$) more abundant in RM cheeses at 3 months, but not at 6 months ripening. The content of toluene and xylenes tended to increase with ripening time. Trimethyl pyrazine, the only nitrogen compound found in the headspace of Manchego cheese, increased significantly during ripening ($P < 0{\cdot}05$) in PM cheeses.

Although the differences between PM and RM cheeses were significant for many volatile compounds, the stepwise discriminant analysis could separate 100% of cheeses by using only 16 compounds. The standardized canonical discriminant function coefficients for these 16 variables are listed in Table 5. Some compounds, such as 2,3-butanedione, 2,3-pentanedione, and 3-methyl-1-heptene, were more abundant in PM cheeses, but most were more abundant in RM cheeses.

Sensory analysis

Mean sensory scores for Manchego cheese odour and aroma quality and odour and aroma intensity are given in Table 6. RM cheeses received higher scores than PM cheeses for odour quality, but differences were significant only for 6-month-old

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Table 4. *Relative abundance*[1] (*mean* ± SD) *of terpenes, hydrocarbons, and aromatic, sulphur and nitrogen compounds detected in the volatile fraction of raw milk (RM) and pasteurized milk (PM) Manchego cheese during ripening*

(Values are means ± SD for $n = 16$ or 24)

Volatile compound	3 months: RM cheese ($n = 24$)	3 months: PM cheese ($n = 16$)	6 months: RM cheese ($n = 24$)	6 months: PM cheese ($n = 16$)	9 months: PM cheese ($n = 16$)	Significance of ripening time[2]: RM	Significance of ripening time[2]: PM
Hydrocarbons	2·94 ± 1·86	3·33 ± 3·18	3·26 ± 2·97	7·09 ± 10·96	17·54 ± 48·93	NS	NS
1,3-Pentanediene	0·24 ± 0·50[b]	0·66 ± 1·25[a]	0·48 ± 1·73[b]	3·79 ± 8·61[a]	14·09 ± 46·48	NS	NS
n-Heptane	0·47 ± 0·25[a]	0·31 ± 0·11[b]	0·46 ± 0·23	0·45 ± 0·26	0·36 ± 0·16	NS	*
n-Octane	1·79 ± 0·79[a]	1·27 ± 0·48[b]	1·84 ± 0·68	1·66 ± 0·73	1·64 ± 0·71	NS	NS
3-Methyl-1-heptene	0·44 ± 0·32[b]	1·09 ± 1·34[a]	0·48 ± 0·33[b]	1·19 ± 1·36[a]	1·45 ± 1·58	NS	NS
Terpenes	1·98 ± 5·97	1·33 ± 0·9	1·86 ± 4·89	2·09 ± 1·46	2·47 ± 1·80	NS	NS
α-Pinene	0·46 ± 0·48[b]	0·94 ± 0·53[a]	0·56 ± 0·52[b]	1·62 ± 1·05[a]	1·94 ± 1·36	NS	**
Limonene	0·71 ± 2·40	0·32 ± 0·30	0·65 ± 1·96	0·39 ± 0·34	0·45 ± 0·35	NS	NS
p-Cymene	0·81 ± 3·09	0·07 ± 0·07	0·65 ± 2·41	0·08 ± 0·07	0·08 ± 0·09	NS	NS
Free fatty acids	0·91 ± 0·52	0·90 ± 0·39	1·22 ± 0·59	0·99 ± 0·72	0·75 ± 0·36	NS	NS
Acetic acid	0·44 ± 0·22	0·41 ± 0·18	0·47 ± 0·30	0·41 ± 0·26	0·35 ± 0·14	NS	NS
Butanoic acid	0·47 ± 0·30	0·49 ± 0·21	0·75 ± 0·29	0·58 ± 0·46	0·40 ± 0·22	*	NS
Sulphur compounds	4·25 ± 7·78	3·26 ± 5·93	2·22 ± 2·58	2·56 ± 3·62	2·21 ± 2·89	NS	NS
Carbon disulphide	3·00 ± 6·42	2·48 ± 5·27	0·96 ± 1·12	1·67 ± 2·97	1·17 ± 2·14	*	NS
Dimethyl sulphide	1·03 ± 1·20[a]	0·64 ± 0·57[b]	1·02 ± 1·31	0·66 ± 0·53	0·78 ± 0·59	NS	NS
Dimethyl disulphide	0·22 ± 0·16[a]	0·14 ± 0·09[b]	0·24 ± 0·15	0·23 ± 0·12	0·26 ± 0·16	NS	**
Aromatic compounds	6·13 ± 9·61	4·43 ± 2·99	10·03 ± 11·11	9·00 ± 10·7	7·61 ± 8·87	NS	NS
Toluene	5·67 ± 8·97	3·96 ± 2·56	9·25 ± 10·19	8·28 ± 10·1	7·07 ± 8·42	NS	NS
1,4 Dimethyl benzene (*p*-xylene)	0·08 ± 0·08	0·07 ± 0·08	0·14 ± 0·14	0·12 ± 0·07	0·09 ± 0·08	*	*
1,3 Dimethyl benzene (*m*-xylene)	0·22 ± 0·34	0·30 ± 0·27	0·43 ± 0·62	0·47 ± 0·45	0·33 ± 0·23	*	NS
1,2 Dimethyl benzene (*o*-xylene)	0·16 ± 0·22	0·10 ± 0·08	0·21 ± 0·16[a]	0·13 ± 0·08[b]	0·12 ± 0·14	NS	NS
Nitrogen compounds							
Trimethyl pyrazine	0·006 ± 0·03	ND	0·02 ± 0·07[b]	0·11 ± 0·33[a]	0·25 ± 0·46	NS	*

[1] Relative abundance expressed as percentage of the cyclohexanone peak. [2] Significance of ripening time: NS, $P > 0·05$; * $P \leqslant 0·05$; ** $P \leqslant 0·01$; *** $P \leqslant 0·001$. ND, below detection limit. [ab] Means followed by a different letter within the same ripening time were significantly different ($P < 0·05$).

Table 5. *Standardized canonical discriminant functions coefficients of the 16 variables used in the stepwise discriminant analysis*

Variable	Function 1
2,3-Butanedione	−0·8317
2,3-Pentanedione	−0·3164
3-Methyl-1-heptene	−0·3850
Ethyl acetate	−0·5183
2-Butanol	0·9884
2-Butanone	0·5360
2-Hexanone+2-hexanal	−0·3407
2-Methyl-1-propanol	1·0196
2-Pentanol	0·7545
Propyl hexanoate	1·0803
2-Methyl-propyl hexanoate	−2·1189
2-Nonanone	0·3648
2-Butoxy-ethanol	−1·2838
3-Methyl-propyl octanoate	−0·4411
Butyl glycol acetate	1·4794
Octanol	0·3879

Table 6. *Sensory scores (on a 0–7 points scale) for the quality and intensity of odour and aroma of raw milk (RM) and pasteurized milk (PM) Manchego cheese during ripening*

	3 months		6 months		9 months	Significance of ripening time[1]	
Sensory scores	RM cheese ($n = 24$)	PM cheese ($n = 16$)	RM cheese ($n = 24$)	PM cheese ($n = 16$)	PM cheese ($n = 16$)	RM	PM
Odour quality[1]	4·36±0·91	4·21±0·85	4·47±0·94[a]	4·30±0·93[b]	4·35±0·90	NS	NS
Aroma quality[1]	4·31±1·09	4·33±0·90	4·55±1·06	4·38±0·89	4·45±0·99	**	NS
Odour intensity[1]	4·33±0·99[a]	3·84±1·11[b]	4·73±1·04[a]	4·36±1·05[b]	4·61±1·12	***	***
Aroma intensity[1]	4·89±1·01[a]	4·21±1·28[b]	5·35±0·97[a]	4·72±0·98[b]	5·05±1·01	***	***

[1] Significance of ripening time: NS, $P > 0·05$; ** $P \leqslant 0·01$; *** $P \leqslant 0·001$. [ab] Means followed by a different letter within the same ripening time were significantly different ($P \leqslant 0·05$).

cheeses ($P < 0·05$). No significant differences were found for aroma quality. RM cheeses had significantly higher scores for odour and aroma intensity at all stages of ripening.

RM cheeses had higher scores than PM cheeses for aroma attributes at 6 months ripening: animal (0·89 vs. 0·33), sheepy (0·61 vs. 0·28), pungent (0·93 vs. 0·12) and rancid (0·39 vs. 0·11), and lower scores for descriptors: lactic (0·16 vs. 1·47) and toasted (0·29 vs. 1·35). All aroma attributes intensified significantly ($P < 0·05$) with ripening time, except lactic, which slightly but significantly ($P < 0·05$) decreased in RM cheeses, and rancid that did not change significantly either in PM cheeses or in RM cheeses (data not shown).

Principal component analysis

Principal components (PC) analysis of volatile compounds and sensory attributes were carried out separately for RM and PM cheeses. Correlation coefficients of the variables with the functions are listed in Table 7. The first two PC explained 49·3% of the variance in RM cheeses. Primary and secondary alcohols, esters, 2-methyl ketones and branched chain aldehydes showed high positive correlation coefficients with PC 1, along with aroma intensity, and the descriptors 'pungent' and 'rancid'. Dimethyl disulphide, 2,3-butanedione and aroma quality had negative correlation

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Table 7. *Correlation coefficients for volatile compounds and sensory attributes with the functions in the principal component analysis with Varimax rotation for raw milk (RM) and pasteurized milk (PM) Manchego cheese*

RM cheese

Variance explained	Function 1 26·5 %	Function 2 22·8 %
Volatile compounds		
Propyl and butyl esters	0·889	−0·131
n-Alkanols	0·871	−0·156
2-Alkanols	0·824	−0·382
Branched chain alkyl esters	0·745	0·511
Ethyl esters	0·678	0·395
Branched chain aldehydes	0·670	0·158
2-Methyl ketones	0·644	−0·307
Dimethyl disulphide	−0·408	0·121
2,3-Butanedione	−0·388	0·136
Branched chain alcohols	−0·046	0·876
3-Methyl butyl butyrate	0·167	0·873
2-Butoxy-ethanol	−0·079	0·868
Dimethyl sulphide	−0·025	0·762
Butyl glycol acetate	−0·148	0·753
Sensory attributes		
Pungent	0·614	0·178
Aroma intensity	0·556	0·532
Rancid	0·532	0·174
Family fruits-flowers	−0·093	0·404
Family toasted	−0·362	−0·389
Aroma quality	−0·262	0·388
Sheepy	0·051	0·256
Family lactic	−0·271	0·197

PM cheese

Variance explained	Function 1 24·5 %	Function 2 16·4 %
Volatile compounds		
Ethyl esters	0·789	−0·004
Branched chain alkyl esters	0·720	0·272
2-Alkanols	0·633	−0·001
n-Alkanols	0·615	0·094
Propyl and butyl esters	0·544	0·046
2-Methyl ketones	0·508	0·201
Dimethyl disulphide	0·491	−0·108
Branched chain aldehydes	0·117	0·817
2,3-Butanedione	−0·444	0·625
2-Butoxy-ethanol	0·413	−0·604
Branched chain alcohols	0·269	0·545
Sensory attributes		
Pungent	0·783	−0·067
Sheepy	0·566	−0·125
Aroma quality	0·542	−0·580
Aroma intensity	0·479	0·424
Family toasted	−0·307	0·707
Family lactic	−0·051	−0·472

coefficients with PC1. Branched chain alcohols, 3-methyl-butyl butyrate, 2-butoxy-ethanol, dimethyl sulphide and butyl glycol acetate, showed high correlation coefficients with PC 2, along with aroma intensity and to a lesser extent aroma quality and the flavour family 'fruits-flowers'.

The first two PC explained 40·9% of the variance in PM cheeses. Esters, primary and secondary alcohols, and 2-methyl ketones showed high positive correlation coefficients with PC 1, along with aroma intensity, aroma quality, and the descriptors 'pungent' and 'sheepy'. 2,3-Butanedione had a negative correlation coefficient with PC 1. Branched chain aldehydes and alcohols, and 2,3-butanedione, showed high correlation coefficients with PC 2, along with the aroma descriptor 'toasted'. Aroma quality showed a negative correlation coefficient with this function.

DISCUSSION

The maintenance of the peculiarities of the original product is important to PDO cheeses, in which the wild microbiota from RM is mostly responsible of the peculiar bouquet (Beuvier *et al*. 1997; Ginzinger *et al*. 1999; Lechner *et al*. 1999). In the case of Manchego cheese the use of PM has led to the manufacture of a product with sensory characteristics quite different from those of RM cheese (Gaya *et al*. 1990). In this study, it has been shown that these sensory differences can be correlated, to a certain extent, with the volatile fraction composition.

The volatile fraction of RM Manchego cheese contained most of the compounds reported for other cheese varieties (Bosset & Gauch, 1993; Urbach, 1993; Barbieri *et al*. 1994; Fernández-García, 1996; Molimard & Spinnler, 1996; Engels *et al*. 1997; Moio & Addeo, 1998; Izco & Torre, 2000). Comparison of our results with those obtained for other cheeses is difficult due to the use of different isolation and quantification techniques. Even working on the same cheese variety, Martínez-Castro *et al*. (1991) obtained a different profile of volatile compounds in PM Manchego cheese using the SDE extraction procedure, with fatty acids being quantitatively the main compounds. Villaseñor *et al*. (2000), using a dynamic headspace technique, also obtained a different profile in commercial samples of Manchego cheese, detecting no primary alcohols or diacetyl, not as many ester species, aromatic compounds and terpenes, and more hydrocarbons and branched chain fatty acids than in our study.

As expected, RM cheeses showed higher abundances than PM cheeses for most volatile compounds, and their flavour was stronger. More intense overall aroma has been repeatedly reported for RM cheeses compared with PM cheeses (Gaya *et al*. 1990; McSweeney *et al*. 1993; Beuvier *et al*. 1997; Buchin *et al*. 1998; Ginzinger *et al*. 1999).

Alcohols and esters predominated in the profile of RM Manchego cheeses. According to PCA, aroma intensity showed the highest positive correlation coefficients with esters, branched chain alcohols and branched chain aldehydes ($P < 0{\cdot}001$). This is a consistent result provided the low perception thresholds of these compounds. Meinhart & Schreier (1986) and Barbieri *et al*. (1994) found high concentrations of many species of esters in Parmesan cheese, and Virgili *et al*. (1994) found a high correlation between aroma intensity of Parmigiano-Reggiano cheese, branched chain aldehydes and esters. Esters possess pleasant fruity notes, but in our study, significant positive correlations were found between the descriptor 'pungent' and esters, n-alkanols and branched chain aldehydes. The microorganisms involved

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in ester formation seem to be mainly yeasts, but some bacteria, and chemical reactions are also responsible (Gripon *et al.* 1991). The branched chain aldehydes and alcohols originating from leucine, isoleucine and valine degradation are considered key-flavour components in some RM cheeses like Parmesan, Proosdij (Engels *et al.* 1997), Emmental (Thierry *et al.* 1999), PDO Mahón and Comté cheeses (Bosset & Gauch, 1993), but other authors related aldehydes with malty off-flavours in Cheddar cheese (Dunn & Lindsay, 1985).

In PM Manchego cheeses, 2,3-butanedione (diacetyl) and methyl-ketones were quantitatively important. However, aroma intensity in PM cheeses was correlated with branched chain aldehydes, esters, methyl ketones and 2-alkanols ($P < 0{\cdot}001$), but not with diacetyl. Kubícková & Grosh (1997) identified diacetyl, along with 3-methyl-butanal, as a potent odorant in Camembert cheese.

Since concentrations of 2-methyl ketones and branched chain aldehydes were higher in PM cheeses than in RM cheeses or no significant difference was found, corresponding higher concentrations of the reduction products 2-alkanols and branched chain alcohols would have been expected. However, alcohols were much more abundant in RM than in PM cheeses. The activity of the indigenous microbiota would have led in RM cheeses to the reduction of carbonyls in the corresponding alcohols, either by enzymatic reactions or indirectly by reducing the redox potential. Buchin *et al.* (1998) obtained similar results in RM and PM Morbier-type cheeses.

Correlation coefficients between aroma quality and volatile compounds were generally low. In RM cheeses, aroma quality showed a positive correlation ($P < 0{\cdot}05$) with isoamyl butyrate, 2-butoxyethanol, branched chain alcohols and dimethyl sulphide, while a negative correlation ($P < 0{\cdot}01$) with 2-alkanols. In PM cheeses, aroma quality was positively correlated with ethyl esters, methyl ketones, n-alkanols, 2-butoxyethanol, ($P < 0{\cdot}01$) and with dimethyl disulphide ($P < 0{\cdot}05$), and negatively correlated ($P < 0{\cdot}001$) with diacetyl, possibly because the high concentrations of this compound may have disturbed the flavour balance of traditional Manchego cheese. Diacetyl is a major aromatic compound in fermented milk and fresh cheese (Cogan, 1995), and contributes to the flavour of many cheeses. A high significant correlation ($P < 0{\cdot}001$) was found between diacetyl, branched chain aldehydes, and the aroma family 'toasted' (also described as 'caramel'), which was very strong in PM cheeses. Diacetyl aroma is usually defined as buttery, but also vanilla, and in this case it could be partly responsible for the toasted flavour. Diacetyl is reduced to acetoin and 2,3-butylene-glycol by starter cultures, and, in RM cheeses, further to 2-butanone and 2-butanol, probably by the action of non-starter bacteria (Keen *et al.* 1974). High amounts of the last two compounds were present in RM but not in PM Manchego cheeses.

Both aroma quality and aroma intensity had positive coefficients for PC1 in PM cheeses, but in RM cheeses, aroma intensity had a positive coefficient and aroma quality a negative coefficient for PC1. This could be indicating that in a mild PM cheese panellists considered flavour intensity and attributes like 'pungent' as quality enhancers, but in RM cheese, panellists seemed to be underscoring cheeses with more intense flavour notes, maybe associated with off-flavours.

It could be concluded that RM and PM Manchego cheese are in fact two different products from the sensory point of view. The use of commercial starter cultures for PM cheese manufacture with a high proportion of diacetyl producers, together with the lack of wild microbiota, is detrimental for the original sensory characteristics of traditional Manchego cheese, mainly due to the unbalanced high content of diacetyl. An equilibrated amount of diacetyl together with some other

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volatile compounds, such as branched chain alcohols and esters, seems to result in a better acceptance of RM cheeses, but an excess of any of those compounds causes a quality decrease. The use of autochthonous strains of lactococci as starter cultures or the addition of strains of other lactic acid bacteria as adjunct cultures for PM cheese manufacture could help in the maintenance of the traditional characteristics of Manchego cheese.

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