Lait 82 (2002) 683–698

DOI: 10.1051/lait:2002042

Original article

Evolution of the volatile components of ewe raw milk La Serena cheese during ripening. Correlation with flavour characteristics

María CARBONELL, Manuel NUÑEZ, Estrella FERNÁNDEZ-GARCÍA*

Departamento de Tecnología de Alimentos, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Carretera de La Coruña, km 7, 28040 Madrid, Spain

(Received 25 July 2001; accepted 11 March 2002)

Abstract – La Serena cheese is a soft to semi-soft Spanish variety manufactured from Merino ewe raw milk coagulated with an extract of *Cynara cardunculus*. In the present work a purge and trap extraction procedure coupled to gas chromatography – mass spectrometry was used to study the volatile fraction evolution during ripening of La Serena cheese. A total of 112 compounds were identified. Alcohols were quantitatively the main chemical family in the volatile fraction, increasing significantly during ripening. Ethanol, 1-propanol, 2-propenol, 2-propanol, 2-butanol, 2-pentanol and the branched chain 2-methyl-1-propanol and 3-methyl-1-butanol, were abundant at the end of ripening. Esters, especially ethyl esters of acetic, butanoic, hexanoic and octanoic acids and 3-methyl-1-butanol acetate, were found at high levels in the headspace of La Serena cheese. Concentrations of most esters increased dramatically during ripening, and, given their low perception thresholds, may be considered as key constituents of the aroma of this cheese variety. The principal component analysis of sensory attributes and volatile compounds extracted 3 functions. Linear aldehydes and alcohols, 2-alkanols, branched-chain alcohols, 2-methyl-ketones, alkanes, terpenes and esters other than ethyl esters correlated positively with function 1 (25.8% of the variance explained). Odour and aroma quality, along with the sensory attributes lactic, fruits-flowers, and clean cheese flavour, and the volatiles ethanol and ethyl esters, had a high correlation coefficient with function 2 (14.8% of the variance explained). 2-Butanone and the sensory attributes fishy and putrid had a negative correlation coefficient with this function. Odour and aroma intensity, along with the sensory attributes animal, pungent and fishy, and the volatile compounds branched chain aldehydes, n-alkanols, 2-butanol and esters, were positively correlated with function 3 (11.0% of the variance explained). Terpenes had a negative correlation coefficient with this function.

Volatile compound / ewe raw milk / La Serena cheese / ripening / flavour

---

* Correspondence and reprints
Tel.: (34) 913476771; fax: (34) 913572293; e-mail: fgarcia@inia.es

Résumé – **Évolution des composés volatils dans le fromage La Serena pendant l'affinage. Corrélation avec les caractéristiques sensorielles.** La Serena est un fromage à pâte molle ou demi-dure fabriqué à partir du lait cru de brebis Merino coagulé avec un extrait de *Cynara cardunculus.* Pour étudier la fraction volatile pendant l'affinage du fromage La Serena, un dispositif d'extraction par espace de tête dynamique « purge and trap » couplé à un appareil de CG-SM a été utilisé. Les alcools étaient quantitativement la principale famille chimique dans la fraction volatile, et augmentaient significativement au cours de l'affinage. L'éthanol, le 1-propanol, le 2-propenol, le 2-propanol, le 2-butanol, le 2-pentanol et les alcools ramifiés 2-méthyl-1-propanol et 3-méthyl-1-butanol étaient très abondants en fin d'affinage. Des esters, principalement des esters éthyliques des acides acétique, butanoïque, hexanoïque et octanoïque, ont été trouvés à des niveaux assez élevés dans la fraction volatile du fromage La Serena. Les concentrations de la plupart des esters augmentaient considérablement pendant l'affinage et, compte tenu de leurs seuils bas de perception, ils peuvent être considérés comme des composés clés pour l'arôme de ce fromage. L'analyse en composantes principales des caractéristiques sensorielles et des composés volatils a donné lieu à trois fonctions. Les aldéhydes et les alcools primaires, 2-alkanols, alcools ramifiés, 2-méthyl-cétones, hydrocarbures, terpènes et des esters autres que les esters éthyliques montraient une corrélation positive avec la fonction 1 (expliquant 25,8 % de la variance). La qualité de l'odeur et de l'arôme, avec les descripteurs sensoriels lactique, fruits-fleurs, et arôme franc de fromage, et les composés volatils éthanol et esters éthyliques montraient une corrélation élevée avec la fonction 2 (expliquant 14,8 % de la variance). La 2-butanone et les descripteurs sensoriels poisson et pourri montraient une corrélation négative avec cette fonction. L'intensité de l'odeur et de l'arôme, avec les descripteurs sensoriels animal, piquant et poisson, et les composés volatils aldéhydes ramifiés, n-alkanols, 2-butanol et esters montraient une corrélation positive avec la fonction 3 (expliquant 11,0 % de la variance). Les terpènes avaient un coefficient de corrélation négatif avec cette fonction.

Composé volatil / lait cru de brebis / fromage La Serena / affinage / arôme

1. INTRODUCTION

According to the balance theory, the flavour of cheese is determined by a number of volatile and non-volatile compounds in equilibrium within the aqueous and fat phases [34]. The enzymatic processes occurring on milk proteins, fat, lactose and citrate during cheese ripening give rise to compounds that confer the cheese with unique flavour and texture characteristics. It is generally accepted that the volatile compounds build up the odour and aroma of food. This is the reason why several research groups have undertaken studies on the aroma compounds of different cheese varieties [5, 8, 13, 20, 31, 32]. The volatile fractions of some Spanish cheeses, Manchego [17, 28, 43], Gamonedo [21], Cabrales [19], Afuega'l Pitu [16], Idiazábal [9], Mahón [35] and Roncal [22], have been studied in the last decade as a response to the growing interest in the characterisation of traditional products protected by a designation of origin. Several review articles summarise the present knowledge on volatile formation and significance [10, 18, 29, 33, 38, 41, 42], and on the analytical techniques used for their study [12, 27, 37].

Gas chromatography is the method of choice for volatile compounds analysis, but the isolation of compounds from the cheese matrix can be carried out by different methods, such as high vacuum distillation [31, 32], simultaneous distillation extraction [9, 28], supercritical fluid extraction [25] or headspace techniques [5, 13, 16, 43]. Extraction by dynamic headspace has been found advantageous over other techniques because of its reduced sample preparation time, high sensitivity, and limited risks of artefacts related to the use of solvents [8]. The suitability of the electronic nose for the study of cheese flavour is still being checked [39].

La Serena is a small (14–16 cm diameter, 5–6 cm height) soft to semi-soft cheese, made in Extremadura (western Spain), from Merino ewe raw milk, and protected by a designation of origin. No starter cultures are added, and vegetable rennet, previously obtained from maceration of thistle flowers (*Cynara cardunculus*) in water, is used to coagulate the milk. The use of Merino ewe raw milk and vegetable rennet gives the cheese its peculiar characteristics of flavour and texture. La Serena cheese is consumed with a minimum ripening period of 60 days, but it may ripen for up to 4 months. Some chemical and microbiological characteristics of La Serena cheese have been reported previously [14, 15, 30, 36], showing that cheeses produced using vegetable rennet had a lower moisture content and a higher pH, with proteolysis occurring at a faster rate and lipolysis at a slower rate, as compared with cheeses produced using animal rennet. Softening of cheese texture was considerably more pronounced in vegetable rennet cheese, which also showed significantly higher flavour quality and intensity. This is the first study on the characterisation of the volatile fraction of La Serena cheese, dealing with the evolution of the volatiles during ripening and their correlation with sensory characteristics. A detailed study on the seasonal variability of the volatile compounds will be the object of a following paper, using the data obtained for the 2-month-old cheeses.

2. MATERIALS AND METHODS

2.1. Cheeses

Duplicate batches of Designation of Origin La Serena cheeses were made from 150–300 kg Merino ewe raw milk in three selected artisan dairy farms during the four seasonal periods of the year. No starter cultures were added and thistle extract was the only coagulant used. Cheeses were analysed for volatile compounds at 1, 60 and 120 days of ripening. Two cheeses per batch were taken as duplicate samples for dynamic headspace gas chromatography analysis, making a total of 144 measurements (3 dairies × 4 seasons × 2 cheesemaking trials × 3 ripening times × 2 cheeses). Cheese sectors were wrapped in aluminium foil, vacuum packed and stored at –40 ºC until analysis.

2.2. Purge and trap extraction and GC-MS analysis

The singular soft texture of La Serena cheese made it necessary to optimise sample preparation. The final procedure was as follows: prior to sample preparation vacuum packed cheese sectors defrosted overnight at 5 ºC, and then were kept at room temperature until a temperature of 9 ºC was reached. 15 g cheese were homogenised in an analytical blender with 30 g Na_2SO_4 and 75 µL of an internal standard aqueous solution containing 0.5 mg·mL^{-1} cyclohexanone and camphor (Sigma-Aldrich Química, Alcobendas, Spain). An aliquot (2.25 g) of the mixture was subjected to dynamic headspace using helium (45 mL·min^{-1}), in an automatic purge and trap apparatus (HP 7695, Hewlett-Packard, Palo Alto, CA, USA), at 50 ºC for 20 min, with 10 min of previous equilibrium. Volatile compounds were concentrated in a Tenax trap maintained at 30 ºC and 6.5 psi back pressure, with 0.5 min dry purge, and desorbed for 1 min at 230 ºC directly into the injection port at 220 ºC, with a split ratio of 1:20, and 1.4 mL·min^{-1} He flow.

Gas chromatography was carried out in a GC-MS (HP-6890) apparatus equipped with a capillary column HP Innowax (60 m long; 0.25 mm ID; 0.5 µm film thickness). Chromatographic conditions were as follows: 12.5 min at 45 ºC; 4 ºC·min^{-1} to 114 ºC; 6 min at 114 ºC; 7 ºC·min^{-1} to 143 ºC; 15 ºC·min^{-1} to 240 ºC, He flow: 1 mL·min^{-1}. Total analysis time was

51 min. Detection was performed with the mass spectrometer operating in the scan mode, 2.6 scan·s^{-1}, with electronic impact at 70 eV, and source and quadrupole temperatures of 230 ºC and 150 ºC, respectively. Peak identification was by comparison of retention times and ion spectra from real standards (Sigma-Aldrich Química) and spectra from the Wiley 275 library (Wiley & Sons, Inc., New York, USA). Semiquantification was carried out by sum of characteristic ions abundance, with reference to the cyclohexanone peak.

2.3. Sensory analysis

Fourteen trained panellists tasted the cheeses at 60 and 120 days of ripening for quality and intensity of odour and aroma on a 0 to 7 points scale. Representative slices of a maximum of three cheeses per session were presented in closed individual Petri dishes. Odour was defined as the olfactory sensation felt directly by the nose. Aroma was defined as the olfactory sensation felt the retronasal way upon mastication. A descriptive test was developed for La Serena cheese based on the guidelines of Berodier et al. [6]. Panellists were asked to give a score on a 0 to 3 points scale for the following flavour attributes: the families “lactic”, “fruits-flowers”, “vegetal”, “animal”, “toasty”, and the individual flavour descriptors “clean cheese”, “sheepy”, “pungent”, “rancid”, “fishy” and “chemical product”.

2.4. Statistics

Statistical treatment of the data was performed using the SPSS Win 5.4 program. Analysis of variance ($\alpha = 95\%$) was carried out with ripening time as the main effect. Mean comparisons were performed with the Tukey’s honestly significant difference test. Selected volatile components and sums of related compounds found in 60- and 120-day-old cheeses were used for principal component analysis (PCA) with Varimax rotation to correlate the volatile compounds with sensory characteristics.

3. RESULTS AND DISCUSSION

3.1. Evolution of volatile compounds during ripening

The purge and trap extraction and the chromatographic method allowed the determination of 112 volatile compounds in the headspace of La Serena cheese during ripening. Figure 1 shows the aroma profile of cheeses at 1 and 120 days of ripening. A total of 13 aldehydes, 8 ketones, 24 alcohols, 24 esters, 13 terpenes, 8 hydrocarbons, 11 aromatic compounds, 3 sulphur compounds, 3 nitrogen compounds and 5 free fatty acids were identified. Tables I to V list the relative abundance of the compounds at the three studied ripening periods, together with the chromatographic retention times and the ions used for quantification. Ten compounds were found only in some samples: 2-propenyl butanoate, 2-decenal, phellandrene, terpinene, terpinolene, methyl cyclopentane, tetrahydrofurane, pyridone, 1-nonanol and indole, and have not been listed in the tables.

Most of the compounds identified in the volatile fraction of La Serena cheese have been previously detected in other cheese varieties [5, 9, 13, 17, 22, 32, 33, 38, 41] and were present both in 60- and 120-day-old cheeses. The volatile fraction of 1-day-old cheeses contained, as expected, low concentrations of most compounds. Aldehydes, 2,3-butanedione, ethanol, some linear and branched-chain alkanols and hydrocarbons were the compounds mainly represented in fresh cheeses. The results for volatile compounds grouped by families follow.

3.1.1. Carbonyl compounds

Table I shows the abundance of aldehydes and ketones in the volatile fraction of

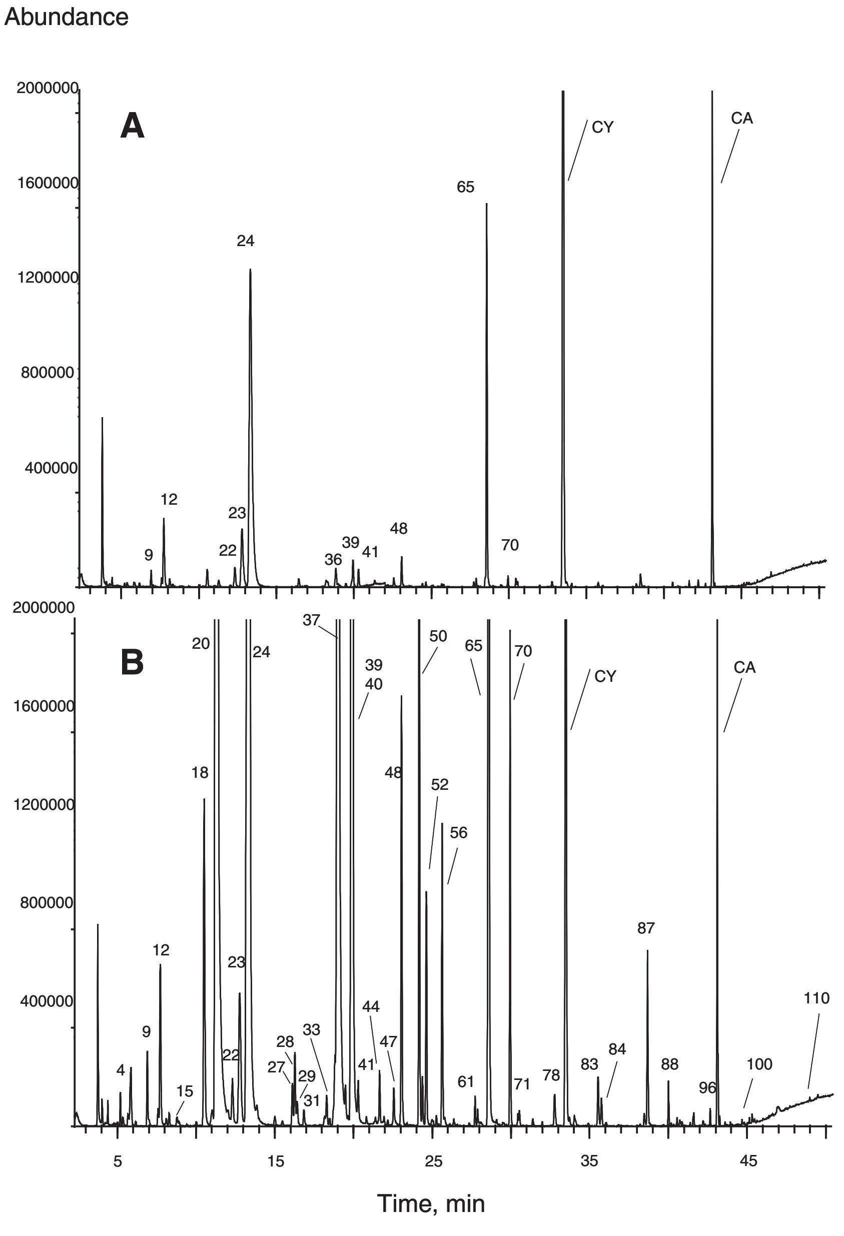

Figure 1. Chromatographic profile corresponding to the headspace of (A) 1-day-old and (B) 120-day-old La Serena cheese made in spring. Identification of peaks as in Tables I to V. CY: internal standard cyclohexanone; CA: internal standard camphor. See Materials and Methods for chromatographic conditions.

La Serena cheese. Aldehydes, unstable compounds that are reduced to alcohols or oxidised to acids, appear at low concentrations in the volatile fraction of most cheeses. However, they have been found as major compounds in mature Proosdij and Parmesan cheeses [13] and in cheeses made using wild strains of *Lactococcus lactis* as starter cultures [4]. All linear aldehydes, with the exception of n-hexanal, increased significantly during ripening. Concentrations of branched-chain aldehydes, especially of 3-methyl-1-butanal, were higher in the fresh cheese, decreased during the first two months (Tab. I), and then increased again until day 120. Their pattern of behaviour during ripening may be due to the different redox conditions in fresh and mature cheeses. 2-Propenal (acroleine) increased significantly during the ripening period. It has been detected in the volatile fraction of Domiati [11] and Roncal [22] cheeses.

Diacetyl (2,3-butanedione), with its sweet buttery and vanilla aroma [33], and, to a lesser extent, its reduction product acetoine, play an important role in the volatile fraction of a number of fermented dairy products. Because of its low perception threshold, diacetyl could be contributing to the flavour of La Serena cheese. Enzymes from the raw milk microbiota further reduce acetoin to 2-butanone and 2-butanol [24, 41], compounds that have been found at high concentrations in La Serena cheese, as well as in other raw milk cheeses [17, 22].

Ketones are intermediate compounds, which may be reduced to secondary alcohols. 2-Butanone and 2-propanone were quantitatively significant among the 2-alkanones in La Serena cheese (Tab. I). However, 2-pentanone and 2-heptanone, although appearing at lower concentrations, are very probably playing a more important role in the aroma due to their much lower perception thresholds. Methyl ketones, particularly 2-heptanone and 2-nonanone, are primarily recognised for their contribution to the flavour of mould-ripened cheeses [13, 20, 32, 38]. 2-Pentanone may impart an orange-peel aroma to cheese [2]. All ketones increased their concentration over the ripening period (Tab. I).

3.1.2. Alcohols

The strong reducing conditions in cheese favour the formation of alcohols from aldehydes and ketones, following reaction pathways which involve alcohol dehydrogenases [33]. Alcohols are quantitatively the main chemical family found in the volatile fraction of La Serena cheese, increasing significantly but at different rates during the ripening process (Tab. II). Ethanol and 3-methyl-1-butanol were the main volatiles in fresh cheese (Fig. 1). Among the 23 species of alcohols quantified, ethanol, 1-propanol, 2-propenol, 2-propanol and 2-butanol, reduction products of acetaldehyde, 1-propanal, 2-propenal, 2-propanone and 2-butanone, respectively, were most abundant at the end of ripening, but very probably not the most important for the aroma, due to their high detection thresholds. 2-Pentanol and the branched chains 2-methyl-1-propanol and 3-methyl-1-butanol, with lower detection thresholds, were also very abundant, and are thus very probably playing an important role in the aroma of this variety. 3-Methyl-1-butanol, as well as 2-methyl-1-propanol, have fruity [23, 38], fusel oil, or whisky odours [31] or even chocolate flavour [3, 4], according to different authors and maybe depending on their concentration. Coming from amino acid catabolism [10] the production of 3-methyl-1-butanol and 3-methyl-1-butanal could be favoured in La Serena cheeses by the use of the highly proteolytic vegetable rennet. 3-Methyl-1-butanol is also a major alcohol in Proosdij cheese [13].

3.1.3. Esters

Twenty-three species of esters were quantified in the volatile fraction of La

Table I. Relative abundance (mean ± SD) of the carbonyl compounds detected in the volatile fraction of La Serena cheese during ripening.

PN	Carbonyl compound	RT	QI	1 d n = 48	60 d n = 48	120 d n = 48
	Aldehydes			2.80 ± 1.75 ^{b}	3.62 ± 1.93 ^{b}	6.68 ± 4.45 ^{a}
5	Acetaldehyde	5.39	44,43,42,41	0.84 ± 0.46 ^{c}	1.22 ± 0.42 ^{b}	1.55 ± 0.46 ^{a}
10	n-Propanal	7.05	58,57	0.18 ± 0.07 ^{b}	0.19 ± 0.12 ^{b}	0.54 ± 0.55 ^{a}
15	2-Propenal	8.82	56,55,53	0.05 ± 0.04 ^{c}	0.83 ± 0.80 ^{b}	2.70 ± 2.60 ^{a}
17	n-Butanal	10.02	44,72,57,	0.08 ± 0.05 ^{c}	0.12 ± 0.06 ^{b}	0.20 ± 0.07 ^{a}
30	n-Pentanal	16.47	58,57,55	0.11 ± 0.03 ^{b}	0.14 ± 0.05 ^{a}	0.15 ± 0.06 ^{a}
47	n-Hexanal	22.57	44,56,41,72	0.92 ± 0.80 ^{a}	0.53 ± 0.30 ^{a}	0.69 ± 0.40 ^{a}
62	n-Heptanal	27.80	70,44,57,55	0.15 ± 0.08 ^{b}	0.15 ± 0.05 ^{b}	0.24 ± 0.11 ^{a}
86	n-Nonanal	38.48	57,56,98,70	0.26 ± 0.10 ^{b}	0.23 ± 0.07 ^{b}	0.31 ± 0.10 ^{a}
93	n-Decanal	42.21	57,55,70,82	0.21 ± 0.12 ^{b}	0.21 ± 0.06 ^{b}	0.30 ± 0.10 ^{a}
	Branched chain aldehydes			6.97 ± 6.1 ^{a}	1.75 ± .0.80 ^{b}	2.92 ± 1.52 ^{b}
11	2-Methyl-propanal	7.60	43,41,72,39	0.95 ± 0.50 ^{a}	0.72 ± 0.20 ^{b}	0.92 ± 0.20 ^{a}
21	2-Methyl-1-butanal	12.05	57,58,41,39	0.29 ± 0.20 ^{a}	0.23 ± 0.16 ^{a}	0.36 ± 0.46 ^{a}
22	3-Methyl-1-butanal	12.33	44,41,58,71	5.73 ± 5.40 ^{a}	0.8 ± 0.44 ^{b}	1.64 ± 0.86 ^{b}
	Ketons (sum except peak 20)			8.15 ± 4.86 ^{b}	10.74 ± 7.01 ^{b}	22.51 ± 16.78 ^{a}
12	2-Propanone	7.77	43,58	7.50 ± 4.30 ^{b}	8.60 ± 4.90 ^{b}	12.70 ± 6.60 ^{a}
20	2-Butanone	11.28	43,72,57,42	1.15 ± 0.45 ^{c}	216 ± 323 ^{b}	532 ± 440 ^{a}
28	2-Pentanone	16.31	43,86,71,58	0.19 ± 0.12 ^{b}	0.83 ± 0.98 ^{b}	5.51 ± 6.50 ^{a}
35	3-Methyl-pentanone	18.73	43,57,72,41	0.03 ± 0.07 ^{b}	0.49 ± 0.44 ^{a}	0.43 ± 0.33 ^{a}
61	2-Heptanone	27.74	43,58,71,114	0.33 ± 0.17 ^{b}	0.66 ± 0.51 ^{b}	3.50 ± 3.00 ^{a}
85	2-Nonanone	38.21	58,43,71,142	0.10 ± 0.20 ^{b}	0.16 ± 0.10 ^{b}	0.37 ± 0.35 ^{a}
	Diketons			3.22 ± 2.30 ^{b}	4.11 ± 1.63 ^{ab}	6.45 ± 5.38 ^{a}
29	2,3-Butanedione	16.46	43,86	2.02 ± 1.80 ^{b}	2.40 ± 1.10 ^{ab}	3.05 ± 2.18 ^{a}
78	3-Hydroxy-2-butanone	32.70	45,43,88,73	1.20 ± 1.50 ^{b}	1.70 ± 1.53 ^{b}	3.40 ± 3.20 ^{a}

Relative abundance as percentage of the cyclohexanone peak. PN: peak number in the chromatogram; RT: retention time; QI: ions used for quantification. ^{abc} Means followed by the same letter within the same row are not significantly different ($P > 0.05$).

Table II. Relative abundance (mean ± SD) of alcohols detected in the volatile fraction of La Serena cheese during ripening.

PN	Alcohols	RT	QI	1 d n = 48	60 d n = 48	120 d n = 48
	n-Alkanols (sum except ethanol)			5.07 ± 3.16^{c}	84.43 ± 74.51^{b}	240 ± 157^{a}
24	Ethanol	13.40	45,46	181 ± 121^{b}	421 ± 127^{a}	436 ± 136^{a}
39	1-Propanol	19.90	59,42,60,41	2.83 ± 1.90^{c}	57.9 ± 49.7^{b}	189 ± 121^{a}
56	1-Butanol	25.67	56,41,43,39	0.33 ± 0.12^{c}	20.7 ± 19.0^{b}	45.3 ± 30.8^{a}
71	1-Pentanol	30.47	55,70,57	0.94 ± 0.39^{b}	0.85 ± 0.31^{b}	1.24 ± 0.60^{a}
84	1-Hexano1	35.77	56,55,43,69	0.46 ± 0.47^{b}	1.65 ± 1.45^{a}	2.25 ± 1.60^{a}
87	2-Butoxy-ethanol	38.68	57,45,41,87	0.14 ± 0.07^{b}	2.90 ± 3.90^{a}	2.30 ± 3.00^{a}
89	1-Heptanol	40.55	70,56,55,69	0.22 ± 0.14^{b}	0.24 ± 0.09^{b}	0.33 ± 0.15^{a}
97	1-Octanol	43.27	56,69,84,55	0.15 ± 0.07^{b}	0.19 ± 0.06^{b}	0.30 ± 0.16^{a}
	2-Alcanols (sum except 2-butanol)			10.01 ± 4.27^{c}	28.5 ± 15.3^{b}	63.2 ± 49.5^{a}
23	2-Propanol	12.78	45,43	9.40 ± 3.80^{c}	22.0 ± 9.70^{b}	37.0 ± 27.0^{a}
37	2-Butanol	19.00	45,59,43,41	1.16 ± 0.99^{c}	294 ± 359^{b}	1038 ± 646^{a}
51	2-Pentanol	24.44	45,55,73	0.36 ± 0.20^{c}	5.27 ± 4.70^{b}	23.3 ± 20.4^{a}
63	2-Hexanol	28.11	45,69,56,41	0.07 ± 0.04^{c}	0.16 ± 0.09^{b}	0.38 ± 0.25^{a}
79	2-Heptanol	33.74	45,55,83,70	0.17 ± 0.23^{c}	0.92 ± 0.70^{b}	2.38 ± 1.80^{a}
94	2-Nonanol	42.34	45,69,55,70	0.01 ± 0.00^{b}	0.10 ± 0.10^{a}	0.10 ± 0.05^{a}
	Branched chain alcohols			132 ± 125^{b}	175 ± 117^{b}	262 ± 145^{a}
19	2-Methyl-2-propanol	11.00	59,41,57,42	0.21 ± 0.06^{c}	0.65 ± 0.47^{b}	1.04 ± 0.09^{a}
48	2-Methyl-1-propanol	23.05	43,41,42,33	15.0 ± 17.7^{b}	20.4 ± 15.6^{b}	34.0 ± 26.7^{a}
65	3-Methyl-1-butanol	28.60	55,70,42,57	116 ± 106^{b}	154 ± 101^{b}	226 ± 118^{a}
92	2,6-Dimethyl-heptanol	41.58	69,43,57,87	0.41 ± 0.85^{a}	0.21 ± 0.28^{a}	0.22 ± 0.34^{a}
	Unsaturated alcohols					
50	2-Propen-1-ol	24.20	57,39,58,40	0.15 ± 0.15^{c}	30.5 ± 36.4^{b}	90.0 ± 60.3^{a}
58	1-Penten-3-ol	26.38	57,41,67,71	0.30 ± 0.48^{a}	0.12 ± 0.10^{b}	0.20 ± 0.16^{ab}
67	2-Buten-1-ol	29.11	57,72,39,53	0.03 ± 0.05^{b}	0.24 ± 0.15^{a}	0.24 ± 0.15^{a}
72	2-Methyl-3-buten-1-ol	30.55	41,56,68,86	0.41 ± 0.21^{c}	0.65 ± 0.10^{b}	0.90 ± 0.25^{a}
80	3-Methyl-3-buten-1-ol	34.06	71,86,41,53	0.26 ± 0.15^{c}	0.60 ± 0.19^{b}	0.74 ± 0.40^{a}

Relative abundance as percentage of the cyclohexanone peak. [abc] Means followed by the same letter within the same row are not significantly different ($P > 0.05$). PN, RT and QI as in Table I.

Table III. Relative abundance (mean ± SD) of esters detected in the volatile fraction of La Serena cheese during ripening.

PN	Esters	RT	QI	1 d n = 48	60 d n = 48	120 d n = 48
	Ethyl esters			8.47 ± 7.23 [c]	90.0 ± 59.2 [b]	228 ± 128 [a]
18	Ethyl acetate	10.54	43,61,70,88	5.50 ± 4.90 [c]	23.9 ± 9.90 [b]	47.5 ± 24.7 [a]
25	Ethyl propanoate	15.02	57,75,102,74	0.06 ± 0.12 [c]	0.57 ± 0.42 [b]	1.40 ± 0.71 [a]
40	Ethyl butanoate	19.99	59,42,60,41	2.02 ± 1.50 [c]	36.0 ± 26.5 [b]	100 ± 55.6 [a]
44	Ethyl 3-methyl butanoate	21.70	88,85,57,60	0.001 ± 0.00 [b]	0.26 ± 0.20 [b]	1.36 ± 1.00 [a]
27	Ethyl 2-methyl propanoate	15.53	43,71,88,116	0.01 ± 0.01 [c]	0.13 ± 0.10 [b]	0.42 ± 0.30 [a]
54	Ethyl pentanoate	25.28	88,85,57,60	0.001 ± 0.005 [c]	0.29 ± 0.23 [b]	0.85 ± 0.64 [a]
70	Ethyl hexanoate	29.95	88,99,43,60	0.63 ± 0.44 [c]	21.3 ± 16.5 [b]	59.6 ± 33.1 [a]
82	Ethyl heptanoate	34.96	88,43,101,113	0.01 ± 0.05 [c]	0.15 ± 0.14 [b]	0.48 ± 0.44 [a]
83	Ethyl lactate	35.57	45,75,103	0.12 ± 0.11 [c]	3.50 ± 1.70 [b]	5.60 ± 4.00 [a]
88	Ethyl octanoate	40.02	88,101,127,70	0.11 ± 0.09 [c]	3.8 ± 3.40 [b]	10.5 ± 7.00 [a]
96	Ethyl nonanoate	42.84	88,101,141,73	ND	0.02 ± 0.03 [b]	0.06 ± 0.06 [a]
100	Ethyl decanoate	44.84	88,101,157,73	0.01 ± 0.01 [c]	0.11 ± 0.10 [b]	0.20 ± 0.20 [a]
	Other esters			0.35 ± 0.23 [c]	1.04 ± 1.19 [b]	3.51 ± 2.77 [a]
13	Methyl acetate	8.13	43,74,59	0.23 ± 0.12 [b]	0.29 ± 0.19 [ab]	0.40 ± 0.40 [a]
27	Propyl acetate	16.16	43,61,73,59	0.012 ± 0.02 [c]	0.30 ± 0.30 [b]	1.76 ± 1.30 [a]
81	Propyl hexanoate	34.14	99,117,61	0.004 ± 0.02 [b]	0.10 ± 0.40 [b]	0.37 ± 0.35 [a]
45	Butyl acetate	21.97	43,56,73,61	0.08 ± 0.05 [c]	0.26 ± 0.20 [b]	0.73 ± 0.47 [a]
68	Butyl butanoate	29.30	71,56,89	0.01 ± 0.01 [b]	0.03 ± 0.05 [b]	0.11 ± 0.12 [a]
75	Hexyl acetate	31.66	43,56,61,69	0.01 ± 0.01 [c]	0.06 ± 0.05 [b]	0.14 ± 0.13 [a]
	Branched chain esters			0.21 ± 0.15 [c]	8.01 ± 7.46 [b]	18.8 ± 16.6 [a]
31	1-Methyl-propyl acetate	16.88	87,73,61,101	ND	0.06 ± 0.08 [b]	0.56 ± 0.65 [a]
34	2- Methyl propyl acetate	18.48	43,56,73,41	0.07 ± 0.06 [c]	0.27 ± 0.19 [b]	0.58 ± 0.59 [a]
52	3-Methyl-1-butyl acetate	24.64	43,70,55,87	0.00 ± 0.00 [c]	7.10 ± 6.70 [b]	16.46 ± 14.70 [a]
67	1-Methoxy-2-propyl acetate	29.48	43,72,58,87	0.13 ± 0.07 [b]	0.23 ± 0.13 [a]	0.17 ± 0.06 [b]
74	3-Methyl-1-butyl butanoate	31.40	71,70,89,55	0.01 ± 0.02 [c]	0.35 ± 0.36 [b]	1.02 ± 0.56 [a]

Relative abundance as percentage of the cyclohexanone peak. [abc] Means followed by the same letter within the same row are not significantly different ($P > 0.05$). ND: not detected. PN, RT and QI as in Table I.

Serena cheese (Tab. III). The large amounts of ethanol detected at all ripening periods may not be important for the aroma, but ethanol is crucial for the formation of ethyl esters, very abundant in this variety, especially ethyl esters of acetic, butanoic, hexanoic and octanoic acids. The acetate of 3-methyl-1-butanol also reached notable levels in 120-day-old cheeses, due to the large amounts of this branched-chain alcohol present. Concentrations of most esters increased dramatically during ripening. In fact, these compounds were the most abundant family, after alcohols, in the headspace of La Serena cheese. Given their low detection thresholds and the way their concentrations increase with ripening time, esters may be considered as key constituents of the aroma of this variety. Esters are main components of the aroma of Parmesan cheese [5], but an excessive concentration has been reported to cause a defect in Cheddar cheese [7].

Ethyl esters may contribute to the cheese aroma not only by providing fruity notes but also by minimising the harsh flavours produced by other volatile compounds according to Anderson and Day [1]. We will further refer to this masking effect of ethyl esters in La Serena cheese. Low levels of methyl, propyl, butyl and some branched-chain alkyl esters of short-chain fatty acids, and traces of ethyl esters of n-C9 and C10 fatty acids were also detected in this variety.

3.1.4. Miscellaneous compounds

A high concentration and diversity of terpenes were observed in La Serena cheese (Tab. IV), presumably coming either from animal feed or from the thistle used as coagulant. Some of them, α-pinene, camphene, δ-carene, limonene and cymene, seemed to increase during ripening but the variability between dairies was too high to make that increase significant. Larsen [26] has observed the production of terpenes by fungi. All hydrocarbons, coming from lipid oxidation, increased slightly but significantly during ripening (Tab. IV). Hydrocarbons have been frequently reported in the volatile fraction of cheeses [40], although usually at low concentrations not detectable by olfactometry [2].

Aromatic compounds and free fatty acids did not change significantly during ripening (Tab. V). This unexpected result may be due to the inefficacy of the purge and trap technique for the determination of FFA. Three sulphur compounds were detected in La Serena headspace: carbon disulphide, dimethyl sulphide and dimethyl disulphide, with a high variability between dairies. Concentrations of carbon disulphide and dimethyl disulphide increased significantly during ripening, while that of dimethyl sulphide decreased slightly (Tab. V). Two nitrogen compounds were found, acetonitrile and pyrrol, which did not vary significantly during ripening. Their origin is unknown, and they probably were contaminants. Only traces of indole, a compound possessing an offensive odour, were found.

3.2. Sensory analysis. Principal component analysis

Analysis of variance showed significant differences in odour and aroma intensity between 60-day-old and 120-day-old cheeses, but not in odour or aroma quality (Tab. VI). Some aroma attributes scores (on a 0 to 3 points scale) increased significantly from 60 days to 120 days of ripening: 0.35 to 0.50 for the family "lactic", 0.91 to 1.39 for the family "animal", 0.40 to 0.52 for the descriptor "rancid" and 0.43 to 0.69 for the descriptor "pungent".

Due to the complexity of cheese flavour and the limitations of both instrumental methodology and human tasting panels, it is not an easy task to correlate the presence or concentration of volatile compounds with sensory characteristics. However, it was the final aim of our study on the volatile fraction of La Serena cheese, and with that purpose we subjected several groups of volatile

Table IV. Relative abundance (mean ± SD) of terpenes and hydrocarbons detected in the volatile fraction of La Serena cheese during ripening.

PN	Volatile compounds	RT	QI	1 d n = 48	60 d n = 48	120 d n = 48
	Terpens			0.79 ± 1.23	5.09 ± 10.3	9.02 ± 17.1
36	α-Pinene	18.83	93,77,121,91	0.51 ± 0.83	3.57 ± 6.74	6.69 ± 12.6
42	Fenchene	20.99	93,79,121,136	0.02 ± 0.04	0.05 ± 0.10	0.07 ± 0.11
43	Camphene	21.40	93,121,79,136	0.02 ± 0.05	0.25 ± 0.52	0.47 ± 0.99
49	β-Pinene	23.63	93,69,41	0.01 ± 0.03	0.08 ± 0.14	0.09 ± 0.19
55	Thujol	25.46	109,91,67,138	ND	0.07 ± 0.15	0.14 ± 0.29
57	δ-Carene	25.93	93,77,121,136	0.01 ± 0.04	0.60 ± 1.90	0.92 ± 2.10
64	Limonene	28.47	68,93,121,136	0.08 ± 0.12	0.14 ± 0.22	0.23 ± 0.26
66	Eucaliptol	28.98	81,107,139	0.01 ± 0.01	0.02 ± 0.02	0.02 ± 0.03
76	Cymene	31.99	119,134,91,115	0.06 ± 0.09	0.24 ± 0.45	0.33 ± 0.60
107	Endo borneol	46.00	95,110,139,71	0.07 ± 0.02	0.07 ± 0.02	0.06 ± 0.02
	Hydrocarbons			3.23 ± 2.31 [b]	4.88 ± 4.79 [b]	8.83 ± 6.69 [a]
1	n-Pentane	4.03	43,41,57,72	0.33 ± 0.18 [ab]	0.23 ± 0.13 [b]	0.40 ± 0.26 [a]
2	n-Hexane	4.42	57,43,41,86	0.60 ± 0.30 [b]	0.72 ± 0.47 [b]	1.15 ± 0.68 [a]
4	n-Heptane	5.20	43,71,57,100	0.36 ± 0.27 [b]	0.35 ± 0.23 [b]	0.80 ± 0.57 [a]
9	n-Octane	6.97	43,85,57,41	1.53 ± 1.03 [b]	1.98 ± 1.15 [b]	3.50 ± 2.00 [a]
14	3-Methyl-1-heptene	8.31	55,41,70,83	0.19 ± 0.15 [b]	0.30 ± 0.24 [ab]	0.38 ± 0.28 [a]
33	3,7-Dimethyl-2-octene	18.30	70,55,41,140	0.19 ± 0.32 [b]	1.02 ± 2.00 [ab]	2.10 ± 2.00 [a]
38	1,3-Octadiene	19.50	41,55,67,82	0.03 ± 0.06 [b]	0.28 ± 0.57 [ab]	0.50 ± 0.90 [a]

Relative abundance as percentage of the cyclohexanone peak. [abc] Means followed by the same letter within the same row are not significantly different ($P > 0.05$). ND: not detected. PN, RT and QI as in Table I.

Table V. Relative abundance (mean ± SD) of aromatic, sulphur and nitrogen compounds detected in the volatile fraction of La Serena cheese during ripening.

PN	Volatile compounds	RT	QI	1 d n = 48	60 d n = 48	120 d n = 48
	Aromatic compounds			2.31 ± 1.29	3.75 ± 3.45	3.95 ± 2.99
41	Toluene	20.31	91,92,65	1.81 ± 1.10	3.00 ± 2.75	3.10 ± 2.60
53	Ethyl benzene	25.00	91,106	0.07 ± 0.05	0.09 ± 0.08	0.10 ± 0.05
106	Ethyl benzoate	45.64	105,77,122,150	0.01 ± 0.01	0.03 ± 0.03	0.06 ± 0.08
108	Benzene methanol	46.61	121,43,77	ND	0.18 ± 0.08	0.21 ± 0.10
109	Naphtalene	46.91	128,127,129	0.11 ± 0.04	0.12 ± 0.05	0.11 ± 0.05
111	Ethyl 1,2-benzene carboxylate	48.85	90,177	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.02
112	Phenol	49.50	94,66	0.04 ± 0.01	0.06 ± 0.40	0.05 ± 0.02
104	Phenylacetaldehyde	45.34	91,92,65,120	0.14 ± 0.05	0.13 ± 0.03	0.18 ± 0.04
105	Acetophenone	45.50	105,77,120,51	0.12 ± 0.02	0.12 ± 0.02	0.12 ± 0.03
	Free fatty acids			0.08 ± 0.11	0.49 ± 0.97	0.63 ± 0.46
90	Acetic acid	40.80	60,43,45	0.02 ± 0.06	0.06 ± 0.60	0.09 ± 0.08
98	2-Methyl-propanoic acid	43.61	43,73,88,41	0.01 ± 0.02	0.11 ± 0.15	0.10 ± 0.09
99	Butanoic acid	44.69	60,73,55,42	0.02 ± 0.01	0.12 ± 0.09	0.21 ± 0.15
103	3-Methyl-butanoic acid	45.27	60,87,41,74	ND	0.10 ± 0.09	0.13 ± 0.10
110	Hexanoic acid	47.41	60,73,87	0.03 ± 0.02	0.10 ± 0.04	0.10 ± 0.04
	Sulphur compounds			1.08 ± 0.96 [b]	1.84 ± 3.23 [b]	4.75 ± 5.18[a]
7	Carbon disulphide	5.85	76,78,77	0.35 ± 0.30 [b]	1.13 ± 2.50 [b]	3.84 ± 3.97[a]
8	Dimethyl sulphide	6.20	62,47,45,35	0.60 ± 0.61	0.40 ± 0.56	0.45 ± 0.85
46	Dimethyl disulphide	22.20	94,79,45,61	0.13 ± 0.05 [c]	0.31 ± 0.17 [b]	0.46 ± 0.36 [a]
	Nitrogen compounds			1.05 ± 0.38	1.23 ± 0.72	1.75 ± 1.23
32	Acetonitrile	18.18	41,40,39,38	0.82 ± 0.32	0.96 ± 0.65	1.44 ± 1.16
95	Pyrrol	42.69	67,39	0.23 ± 0.06	0.27 ± 0.07	0.31 ± 0.07

Relative abundance as percentage of the cyclohexanone peak. [abc] Means followed by the same letter within the same row are not significantly different ($P < 0.05$). ND: not detected. PN, RT and QI as in Table I.

Table VI. Sensory scores on a 0 to 7 points scale for the quality and intensity of odour and aroma of La Serena cheese at 60 and 120 days of ripening.

Sensory scores	60 d	120 d
Odour quality	4.35 ± 0.37[a]	4.29 ± 0.63[a]
Aroma quality	4.21 ± 0.53[a]	4.25 ± 0.69[a]
Odour intensity	4.55 ± 0.48[b]	4.96 ± 0.33[a]
Aroma intensity	4.64 ± 0.68[b]	5.30 ± 0.36[a]

compounds to Principal Component Analysis, along with certain individual compounds, and scores for odour and aroma quality, for odour and aroma intensity and for some sensory attributes. Table VII lists the correlation coefficients of variables with the three functions extracted.

Function 1 explained 25.8% of the variance and it seemed to be composed only of volatile compounds. Linear aldehydes and alcohols, 2-alkanols, branched-chain

Table VII. Correlation coefficients for volatile compounds and sensory attributes with the functions in the principal component analysis with Varimax rotation.

	Function 1	Function 2	Function 3
Sensory attributes			
Odour quality	–0.2077	0.7922	–0.0058
Aroma quality	–0.1763	0.7607	0.1846
Odour intensity	0.0215	0.0055	0.7128
Aroma intensity	0.2918	–0.1310	0.6072
Family lactic	0.1020	0.6398	–0.1191
Family fruits-flowers	–0.0840	0.4303	0.0726
Family animal	0.1491	0.2415	0.6475
Clean cheese flavour	–0.3152	0.5714	–0.0958
Chemical flavour	0.5327	–0.1977	–0.3436
Rancid	0.3508	–0.0199	0.3200
Pungent	0.0899	–0.0578	0.7171
Fishy	–0.0022	–0.5950	0.5753
Volatile compounds			
Ketones	0.8138	–0.1252	0.0790
n-Aldehydes	0.7946	0.1598	0.0927
2-Alkanols	0.7930	0.2345	–0.0382
Alkanes	0.6886	0.2135	0.1967
Other esters	0.6748	–0.2037	0.3100
Branched chain esters	0.6713	–0.2513	0.3131
Terpenes	0.6707	–0.0782	–0.4491
n-Alkanols	0.6119	0.1124	0.4668
2-Butanone	0.5858	–0.4581	0.2137
Branched chain alcohols	0.5829	–0.3691	0.3840
Diketones	0.5501	–0.1084	0.0330
Sulphur compounds	0.5081	–0.0694	0.2579
Ethyl esters	0.3269	0.6599	0.2706
Ethyl lactate	–0.0352	0.6110	0.2590
Ethanol	0.0836	0.4683	–0.2437
Branched chain aldehydes	0.1475	–0.0173	0.6545
2-Butanol	0.4050	–0.0876	0.4180

alcohols, 2-methyl-ketones, 2-butanone, alkanes, terpenes and esters other than ethyl esters correlated positively with function 1, along with the sensory attribute chemical product. The correlation matrix showed that the descriptor chemical compound was positively correlated ($P < 0.001$) with linear aldehydes (r = 0.35), ketones (r = 0.45), terpenes (r = 0.38) and 2-butanone (r = 0.45).

Function 2 explained 14.8% of the variance and seemed to be related to cheese quality. Along with odour and aroma quality, the sensory attributes lactic, fruits-flowers, and clean cheese flavour, and the volatiles ethanol and ethyl esters, ethyl lactate included, had a high correlation coefficient with function 2. On the other hand, 2-butanone and the sensory attribute fishy had a negative correlation coefficient with this function. The correlation matrix showed that ethyl esters were positively correlated ($P < 0.01$) with odour quality (r = 0.27) and aroma quality (r = 0.27), and with the attributes fruits-flowers (r = 0.27) and rancid (r = 0.35). Branched-chain esters were negatively correlated with odour quality (r = –0.33) and clean cheese flavour (r = –0.40). Some interactions between volatile compounds could be detected in La Serena cheese. Esters correlated positively with the quality of cheese aroma, while 2-butanone correlated negatively. It was observed that cheeses with high concentrations of 2-butanone received higher aroma quality scores than expected when they contained high amounts of esters. Anderson and Day [1] observed a masking effect of esters on the harsh notes of free fatty acids and amines.

Function 3 explained 11.0% of the variance and seemed to be related to flavour intensity. Along with odour and aroma intensity, the sensory attributes animal, pungent and fishy, and the volatile compounds branched-chain aldehydes, n-alkanols, 2-butanol and, to a lesser extent, esters, were positively correlated with function 3. Terpenes had a negative correlation coefficient with this function. The correlation matrix showed that aroma intensity was positively correlated ($P < 0.001$) with linear aldehydes (r = 0.36), ketones (r = 0.38), branched-chain aldehydes (r = 0.45), primary alcohols (r = 0.31), branched-chain alcohols (r = 0.37) and branched-chain esters (r = 0.34). Branched-chain aldehydes and primary alcohols were positively correlated ($P < 0.001$) with the attributes animal (r = 0.39; r = 0.41, respectively) and pungent (r = 0.43). Secondary alcohols were positively correlated ($P < 0.001$) with chemical product flavour (r = 0.34). Branched-chain alcohols and branched-chain esters were positively correlated with rancid (r = 0.36) and pungent flavour (r = 0.36; r = 0.28, respectively) and with odour intensity (r = 0.37), and negatively with clean cheese flavour (r = –0.39) and odour quality (r = –0.31). Methyl, propyl and butyl esters were positively correlated with aroma intensity (r = 0.31) and with rancid flavour (r = 0.34), and negatively correlated with clean cheese flavour (r = –0.39) and odour quality (r = –0.30). Terpenes were positively correlated ($P < 0.001$) with chemical product flavour (r = 0.38).

A high number of volatile compounds could be involved in the odour and aroma of La Serena cheese, as shown by the results obtained in the present work. Especially ethyl esters and some alcohols seem to play a key role in both the aroma intensity and quality of La Serena cheese.

ACKNOWLEDGMENTS

This work was supported by the INIA research project SC98-096. We thank C. Sánchez for her technical assistance and the panellists for the sensory assessments. We thank P. Cavanillas, P. Guisado, A. Gómez and D. Murillo for their kindness and help with the ripening and supply of cheeses.

REFERENCES

[1] Anderson D.F., Day E.A., Quantitation, evaluation and effect of certain microorganisms on flavor components of Blue cheese, J. Agric. Food Chem. 14 (1966) 241–245.

[2] Arora G., Cormier F., Lee B., Analysis of odour-active volatiles in Cheddar cheese headspace by multidimensional GC/MS/sniffing, J. Agric. Food Chem. 43 (1995) 748–752.

[3] Ayad E.A., Verheul A., de Jong C., Wouters J.T.M., Smit G., Flavour forming abilities and amino acid requirements of *Lactococcus lactis* strains isolated from artisanal and non-dairy origin, Int. Dairy J. 9 (1999) 725–735.

[4] Ayad E.A., Verheul A., Wouters J.T.M., Smit G., Application of wild starter cultures for flavour development in pilot plant cheese making, Int. Dairy J. 10 (2000) 169–179.

[5] Barbieri G., Bolzoni L., Careri M., Manglia A., Parolari G., Spagnoli S., Virgili R., Study of the volatile fraction of Parmesan cheese, J. Agric. Food Chem. 42 (1994) 1170–1176.

[6] Berodier F., Lavanchy P., Zannoni M., Casals J., Herrero L., Adamo C., Guide for the flavour evaluation of hard and semihard cheeses, G.E.C.O.T.E.F.T., Poligny, France (1997).

[7] Bills D.D., Morgan M.E., Libbey L.M., Day E.A., Identification of compounds responsible for fruity flavor defect of experimental Cheddar cheeses, J. Dairy Sci. 48 (1965) 1168–1173.

[8] Bosset J.O., Gauch R., Mariaca R., Klein B., Comparison of the various sample treatments for the analysis of volatile compounds by GC-MS: Application to the Swiss Emmental cheese, Mitt. Gebiete Lebensm. Hyg. 86 (1995) 672–698.

[9] Carbonell M., Characterisation of the Idiazábal cheese volatile fraction. Effect of starters and the pasteurisation of the milk. Ph.D. Thesis, Universidad Pública de Navarra, Pamplona, Spain, 1998.

[10] Christensen J.E., Dudley E.G., Pederson J.A., Steele J.L., Peptidases and amino acid catabolism in lactic acid bacteria, Antonie van Leeuwenhoek 76 (1999) 217–246.

[11] Collin S., Osman M., Delcambre S., El Zayat A.I., Dufour J.P., Investigation of volatile flavor compounds in fresh and ripened Domiati cheeses, J. Agric. Food Chem. 41 (1993) 1659–1663.

[12] Delahunty C.M., Piggot J.R., Current methods to evaluate contribution and interactions of components to flavour of solid foods using hard cheese as an example, Int. J. Food Sci. Technol. 30 (1995) 555–570.

[13] Engels W.J.M., Dekker R., de Jong C., Neeter R., Visser S., A comparative study of volatile compounds in the water-soluble fraction of various types of ripened cheese, Int. Dairy J. 7 (1997) 255–263.

[14] Fernández del Pozo B., Gaya P., Medina M., Rodríguez-Marín M.A., Nuñez M., Changes in microflora of La Serena ewes milk cheese during ripening, J. Dairy Res. 55 (1988) 449–455.

[15] Fernández del Pozo B., Gaya P., Medina M., Rodríguez-Marín M.A., Nuñez M., Changes in chemical and rheological characteristics of La Serena ewes milk cheese during ripening, J. Dairy Res. 55 (1988) 457–464.

[16] Fernández-García E., Use of headspace sampling in the quantitative analysis of artisanal Spanish cheese aroma, J. Agric. Food Chem. 44 (1996) 1833–1839.

[17] Fernández-García E., Carbonell M., Nuñez M., Volatile fraction and sensory characteristics of Manchego cheese, 1. Comparison of raw and pasteurised milk cheese, J. Dairy Res. 69 (2002) 574–593.

[18] Fox P.F., Wallace J.M., Formation of flavor compounds in cheese, Adv. Appl. Microbiol. 45 (1997) 17–85.

[19] Frutos M., Sanz J., Martínez-Castro I., Characterization of artisanal cheeses by GC-MS analysis of their medium volatility (SDE) fraction, J. Agric. Food Chem. 39 (1991) 524–530.

[20] Gallois A., Langlois D., New results in the volatile odorous compounds of French cheese, Lait 70 (1990) 89–106.

[21] Gonzalez de Llano D., Ramos M., Polo C., Sanz J., Martinez-Castro I., Evolution of the volatile components of an artisanal blue cheese during ripening, J. Dairy Sci. 73 (1990) 1676–1683.

[22] Izco J.M., Torre P., Characterization of volatile flavour compounds in Roncal cheese extracted by the purge and trap method and analysed by GC-MS, Food Chem. 70 (2000) 409–417.

[23] Karahadian C., Josephson D.B.J., Lindsay R.C., Contribution of *Penicillium* sp. to the flavours of Brie and Camembert cheese, J. Dairy Sci. 68 (1985) 1865–1877.

[24] Keen A.R., Walker J., Peberdy M.F., The formation of 2-butanone and 2-butanol in Cheddar cheese, J. Dairy Res. 41 (1974) 249–257.

[25] Larrayoz P., Carbonell M., Ibañez F., Torre P., Barcina Y., Optimisation of indirect parameters which affect the extractability of volatile aroma compounds from Idiazabal cheese using analytical supercritical fluid extractions (SFE), Food Chem. 64 (1999) 123–127.

[26] Larsen T.O., Volatile flavour production by *Penicillium caseifulvum*, Int. Dairy J. 8 (1998) 883–887.

[27] Mariaca R.G., Bosset J.O., Instrumental analysis of volatile (flavour) compounds in milk and dairy products, Lait 77 (1997) 13–40.

[28] Martínez-Castro I., Sanz J., Amigo L., Ramos M., Martín-Alvarez P., Volatile components of

Manchego cheese, J. Dairy Res. 58 (1991) 239–246.

[29] McSweeney P.L.H., Sousa M.J., Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review, Lait 80 (2000) 293–324.

[30] Medina M., Fernández del Pozo B., Rodríguez-Marín M.A., Gaya P., Nuñez M., Effect of lactic starter inoculation on chemical, microbiological, rheological and sensory characteristics of La Serena cheese, J. Dairy Res. 58 (1991) 355–361.

[31] Moio L., Addeo F., Grana Padano cheese aroma, J. Dairy Res. 65 (1998) 317–333.

[32] Moio L., Piombino P., Addeo F., Odour impact compounds of Gorgonzola cheese, J. Dairy Res. 67 (2000) 273–285.

[33] Molimard P., Spinnler H.E., Review: Compounds involved in the flavor of surface mold-ripened cheeses: origins and properties, J. Dairy Sci. 79 (1996) 169–184.

[34] Mulder H., Taste and flavour forming substances in cheese, Neth. Milk Dairy J. 6 (1952) 157–167.

[35] Mulet A., Escriche I., Rosello C., Tarrazó J., Changes in the volatile fraction during ripening of Mahón cheese, Food Chem. 65 (1999) 219–225.

[36] Nuñez M., Fernández del Pozo B., Rodríguez-Marín M.A., Gaya P., Medina M., Effect of vegetable and animal rennet on chemical, microbiological, rheological and sensory characteristics of La Serena cheese, J. Dairy Res. 58 (1991) 511–519.

[37] Reineccius G.A., Anandaraman S., Analysis of volatile flavours, in: Lawrence J.F. (Ed.), Food Constituents and Food Residues, Marcel Dekker, Basel, Switzerland, 1984, pp. 195–293.

[38] Sablé S., Cottenceau G., Current knowledge of soft cheeses flavour and related compounds, J. Agric. Food Chem. 47 (1999) 4825–4836.

[39] Schaller E., Bosset J.O., Escher F., Practical experience with "electronic nose" systems for monitoring the quality of dairy products, Chimia 53 (1999) 98–102.

[40] Thierry A., Maillard M.B., Le Quéré J.L., Dynamic headspace analysis of Emmental aqueous phase as a method to quantify changes in volatile flavour compounds during ripening, Int. Dairy J. 9 (1999) 453–463.

[41] Urbach G., Relations between cheese flavour and chemical composition, Int. Dairy J. 3 (1993) 389–422.

[42] Urbach G., Contribution of lactic acid bacteria to flavour compounds formation in dairy products, Int. Dairy J. 5 (1995) 877–903.

[43] Villaseñor M.J., Valero E., Sanz J., Martínez-Castro I., Analysis of volatile components of Manchego cheese by dynamic headspace followed by automatic thermal desorption-GC-MS, Milchwissenschaft 55 (2000) 378–382.

To access this journal online:
www.edpsciences.org