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Original article

Seasonal variation of volatile compounds in ewe raw milk La Serena cheese

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Abstract – La Serena cheese is a soft to semi-soft variety made in Extremadura (western Spain) from Merino ewe raw milk using an extract of *Cynara cardunculus* as milk coagulant. A purge and trap apparatus coupled to a GC-MS was used to study the seasonal variation of the volatile fraction of La Serena cheeses made at four artisan dairies. Alcohols and esters were the main chemical families found in La Serena cheese. Most volatile compounds were detected in cheeses made during the whole year, although at different concentrations. Abundances of hydrocarbons, primary alcohols, secondary alcohols of an uneven number of carbon atoms, terpenes, 2,3-butanedione, free fatty acids, nitrogen compounds and aromatic compounds were generally higher in cheeses made during spring. Aldehydes reached the highest levels in spring and summer cheeses. Ethanol and ethyl esters were more abundant in spring and autumn cheeses, while branched chain alcohols and branched chain alkyl esters were more abundant in summer cheeses. Cheeses made in winter showed a lower abundance of most volatile compounds, except dimethyl sulphide and dimethyl disulphide. Cheeses made in spring received the highest scores for odour and aroma quality, and cheeses made in winter received the lowest scores. High amounts of 2,3-butanedione, medium concentrations of most terpenes and alcohols, low levels of methyl ketones, especially of 2-butanone, and very low concentrations of 2-butanol were generally recorded for cheeses with the highest quality scores in sensory evaluation. Concentrations of most ethyl esters and branched chain alkyl esters in the highest scored cheeses were above the mean values in cheeses made during the whole year. However, levels of propyl esters and ethyl esters of branched chain acids were lower in the highest scored cheeses.

Volatile compound / ewe raw milk / La Serena cheese / seasonal variation

Résumé – **Variation saisonnière des composés volatils dans le fromage La Serena au lait cru de brebis.** La Serena est un fromage à pâte molle ou demi-dure d'appellation d'origine contrôlée fait en Extremadura, à l'ouest de l'Espagne, avec du lait cru de brebis Merino et un extrait de *Cynara cardunculus* comme coagulant du lait. Un dispositif de « purge and trap » couplé à un appareil de CG-SM a

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été utilisé pour étudier la variation saisonnière de la fraction volatile des fromages de La Serena fabriqués dans quatre usines pendant une année. Les familles chimiques majoritaires étaient des alcools et des esters. La plupart des composés volatils ont été détectés dans les fromages fabriqués pendant les quatre saisons de l'année, mais à des concentrations différentes. Les concentrations des hydrocarbures, des alcools primaires, des alcools secondaires à nombre impair d'atomes de carbone, terpènes, 2,3-butanedione, acides gras libres, composés azotés et composés aromatiques étaient en général plus élevées dans les fromages de printemps. Les aldéhydes étaient en concentration maximale dans les fromages de printemps et d'été. L'éthanol et les esters éthyliques étaient plus abondants dans les fromages de printemps et d'automne, tandis que les alcools à chaîne ramifiée étaient plus abondants dans les fromages d'été. Les fromages d'hiver montraient une moindre abondance de la plupart des composés volatils, sauf pour le diméthyl sulfure et le diméthyl disulfure. Les fromages de printemps ont obtenu la plus haute note du jury de dégustation pour la qualité de l'odeur et de l'arôme et les fromages d'hiver la plus basse. Des concentrations plus élevées de 2,3-butanedione, des concentrations moyennes de la plupart des terpènes et des alcools, des niveaux plus bas de méthyl-cétones, notamment de 2-butanone, et des niveaux beaucoup plus bas de 2-butanol ont été trouvés pour les fromages les plus appréciés par le jury de dégustation. Les concentrations de la plupart des esters éthyliques et des esters d'alcool à chaîne ramifiée des fromages les plus appréciés étaient plus élevées que les concentrations moyennes de tous les fromages. Cependant, les niveaux des esters propyliques et éthyliques des acides à chaîne ramifiée étaient plus bas dans les fromages les plus appréciés.

Composé volatil / lait cru de brebis / fromage La Serena / variation saisonnière

1. INTRODUCTION

Cheese flavour development is a complex process, in which enzymes from milk, rennet, starter cultures and secondary microbiota are involved, acting on milk proteins, fat and carbohydrates. Season should be considered an important factor influencing the volatile fraction and sensory characteristics of cheeses, especially of those made exclusively from raw milk without the addition of starter cultures such as La Serena cheese, a cheese with a designation of origin. However, not many studies have been reported on this subject [3, 12, 14, 21].

A first study on the volatile fraction of La Serena cheese has been carried out, focusing on the evolution of the volatile compounds during ripening and their correlation with the sensory characteristics [6]. The objective of this paper was to study the seasonal variability of the volatile fraction of La Serena cheese, and to correlate the volatile compounds' abundance with quality and intensity of flavour.

2. MATERIALS AND METHODS

2.1. Cheeses

Cheeses correspond to 60-day-old La Serena cheeses made at four dairy farms as described in a previous paper [6] at the four seasonal periods (spring, summer, autumn and winter) of the year. Two cheeses per batch were taken as duplicate samples for the analysis of the volatile fraction and of cheese sensory characteristics, making a total of 64 measurements (4 dairies × 4 seasons × 2 trials × 2 cheeses).

2.2. Purge and trap and GC-MS

Purge and trap extraction and gas chromatography conditions were as described in a previous work [6].

2.3. Sensory analysis

Cheeses were tasted by 14 trained panellists for quality and intensity of odour and aroma on a 0 to 7 points scale, as described previously [6].

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 season as the main effect. Mean comparisons were performed with the Tukey's honestly significant difference test. Selected volatile components and sums of related compounds were used for discriminant analysis to classify samples by season.

3. RESULTS AND DISCUSSION

3.1. Seasonal variability of volatile compounds

Tables I to V show the relative abundance of the compounds at the four studied seasonal periods, together with the chromatographic retention times and the ions used for quantification. A column has been added showing the main abundance of the compounds in the 10 cheeses with the highest scores for odour and aroma quality (HSC). Other compounds occasionally found in some of the samples, such as 2-propenyl butanoate, 2-decenal, phellandrene, terpinene, terpinolene, methyl cyclopentane, tetrahydrofuran, pyridone, 1-nonanol and indole, were not quantified. The results are presented ordered by chemical families.

3.1.1. Carbonyl compounds

The abundance of aldehydes and ketones in the volatile fraction of La Serena cheese is shown in Table I. Five out of twelve aldehydes and all ketones were significantly different ($P < 0.01$) between dairies (data not shown). Higher concentrations of most aldehydes were found in cheeses made in spring and summer. Acetaldehyde is a product of lactose fermentation and threonine degradation, which has been found in most cheeses. The origin of linear aldehydes is not clear. Certain strains of lactococci can produce branched chain aldehydes, 2-methyl-1-propanal, 2-methyl-1-butanal and 3-methyl-1-butanal, from valine, isoleucine and leucine, respectively, through several enzymatic pathways [1, 2, 7]. 2-Propenal (acrolein) is derived from 3-hydroxypropanal, through the action of some lactic acid bacteria such as *Lactobacillus brevis* and *Lb. coryniformis,* and some anaerobic species are capable of synthesising acrolein from lactic acid [4].

Major ketones in La Serena cheese were 2-butanone and 2-propanone, showing higher concentrations in autumn and summer, respectively. Lower levels of methyl ketones, especially of 2-butanone, were generally recorded for the HSC. Diacetyl (2,3-butanedione) and acetoin (3-hydroxy-2-butanone) were more abundant in spring cheeses. Diacetyl is formed in dairy products from citrate by *L. lactis* ssp. *lactis* var. *diacetylactis* and *Leuconostoc* spp. [8], which have been found to be abundant in La Serena cheese [10]. Relatively higher amounts of 2,3-butanedione were generally recorded for the HSC. Enzymes from bacteria present in raw milk further reduce acetoin to 2-butanone and 2-butanol [15], compounds found at high levels in La Serena cheese (Tabs. I and II) as well as in other raw milk cheeses [11, 14].

3.1.2. Alcohols

At the low redox potential of cheese, aldehydes and ketones are mostly reduced to alcohols (Tab. II). Abundances of 19 alcohols out of 23 were significantly ($P < 0.01$) different between dairies (data not shown). High amounts of ethanol were observed in La Serena cheese in all seasons. Ethanol may be formed from acetaldehyde by leuconostocs and lactobacilli [9] and from lactate by yeasts [17]. High numbers of leuconostocs and relatively high numbers

Table I. Relative abundance (mean ± SD) of the carbonyl compounds detected in the volatile fraction of 60-day-old La Serena cheeses made throughout the year.

Carbonyl compound	RT	QI	Spring n =16	Summer n =16	Autumn n =16	Winter n =16	HSC n =10
n-Alkanals							
Acetaldehyde	5.39	44,43,42,41	1.02 ± 0.18^{b}	1.48 ± 0.37^{a}	1.38 ± 0.61^{a}	0.91 ± 0.20^{b}	1.05 ± 0.21
n-Propanal	7.05	58,57	0.21 ± 0.09^{a}	0.21 ± 0.11^{a}	0.17 ± 0.11^{a}	0.20 ± 0.16^{a}	0.18 ± 0.10
2-Propenal	8.82	56,55,53	1.19 ± 1.12^{a}	1.22 ± 0.53^{a}	0.48 ± 0.44^{b}	0.28 ± 0.27^{b}	0.81 ± 0.80
n-Butanal	10.02	44,72,57,	0.18 ± 0.05^{a}	0.12 ± 0.03^{b}	0.08 ± 0.03^{c}	0.05 ± 0.01^{c}	0.12 ± 0.07
n-Pentanal	16.47	58,57,55	0.17 ± 0.04^{a}	0.09 ± 0.01^{b}	0.10 ± 0.20^{b}	0.15 ± 0.07^{a}	0.15 ± 0.07
n-Hexanal	22.57	44,56,41,72	0.55 ± 0.28^{a}	0.52 ± 0.40^{a}	0.50 ± 0.30^{a}	0.51 ± 0.10^{a}	0.51 ± 0.30
n-Heptanal	27.80	70,44,57,55	0.19 ± 0.05^{a}	0.16 ± 0.04^{b}	0.12 ± 0.02^{c}	0.09 ± 0.04^{c}	0.15 ± 0.05
n-Nonanal	38.48	57,56,98,70	0.25 ± 0.05^{ab}	0.28 ± 0.03^{a}	0.24 ± 0.07^{b}	0.14 ± 0.05^{c}	0.22 ± 0.07
n-Decanal	42.21	57,55,70,82	0.22 ± 0.02^{a}	0.22 ± 0.03^{a}	0.23 ± 0.08^{a}	0.15 ± 0.06^{b}	0.20 ± 0.05
Branched chain aldehydes			1.76 ± 0.40^{b}	2.62 ± 0.86^{a}	1.52 ± 0.15^{b}	1.60 ± 0.94^{b}	1.64 ± 0.62
2 Methyl-1-propanal	7.60	43,41,72,39	0.87 ± 0.19^{a}	0.84 ± 0.11^{a}	0.52 ± 0.03^{b}	0.49 ± 0.19^{b}	0.74 ± 0.21
2-Methyl-1-butanal	12.05	57,58,41,39	0.28 ± 0.04^{a}	0.25 ± 0.05^{ab}	0.22 ± 0.03^{b}	0.10 ± 0.03^{c}	0.25 ± 0.20
3-Methyl-1-butanal	12.33	44,41,58,71	0.61 ± 0.17^{b}	1.53 ± 0.70^{a}	0.78 ± 0.09^{b}	1.01 ± 0.72^{b}	0.65 ± 0.21
Methyl ketones							
2-Propanone	7.77	43,58	9.07 ± 3.35^{ab}	10.52 ± 6.80^{a}	6.53 ± 2.91^{bc}	5.44 ± 0.97^{c}	8.81 ± 6.70
2-Butanone	11.28	43,72,57,42	68.0 ± 143.5^{b}	270 ± 205^{ab}	421 ± 443^{a}	312 ± 457^{ab}	55.80 ± 130
2-Pentanone	16.31	43,86,71,58	0.88 ± 1.05^{ab}	0.71 ± 0.23^{ab}	1.12 ± 1.19^{a}	0.32 ± 0.30^{b}	0.41 ± 0.30
3-Methyl-pentanone	18.73	43,57,72,41	0.77 ± 0.53^{a}	0.24 ± 0.18^{b}	0.40 ± 0.28^{b}	0.43 ± 0.31^{b}	0.49 ± 0.51
2-Heptanone	27.74	43,58,71,114	0.69 ± 0.38^{ab}	0.96 ± 0.57^{a}	0.46 ± 0.36^{b}	0.44 ± 0.32^{b}	0.46 ± 0.31
2-Nonanone	38.21	58,43,71,142	0.19 ± 0.07^{a}	0.17 ± 0.10^{a}	0.14 ± 0.08^{a}	0.19 ± 0.14^{a}	0.14 ± 0.09
Diketones			5.69 ± 3.29^{a}	3.67 ± 1.74^{b}	3.43 ± 1.48^{b}	3.63 ± 1.38^{b}	4.50 ± 3.00
2,3-Butanedione	16.46	43,86	3.14 ± 1.40^{a}	1.76 ± 0.57^{c}	2.18 ± 0.72^{bc}	2.54 ± 0.63^{ab}	2.55 ± 1.20
3-Hydroxy-2-butanone	32.70	45,43,88,73	2.55 ± 1.89^{a}	1.91 ± 1.17^{ab}	1.25 ± 0.76^{b}	1.09 ± 0.75^{b}	1.95 ± 1.80

Relative abundance as percentage of the cyclohexanone peak. RT: retention time; QI: ions used for quantification; HSC: mean concentration of the volatile compound in the 10 highest scored cheeses. [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 60-day-old La Serena cheese made throughout the year.

Alcohol	RT	QI	Spring n =16	Summer n =16	Autumn n =16	Winter n =16	HSC n =10
Primary alcohols							
Ethanol	13.40	45,46	425.5 ± 126.0^{a}	392.0 ± 115.6^{a}	476.0 ± 130.5^{a}	386.0 ± 93.0^{a}	452.0 ± 117.0
1-Propanol	19.90	59,42,60,41	51.61 ± 48.76^{a}	65.55 ± 19.90^{a}	71.41 ± 60.30^{a}	64.18 ± 73.51^{a}	44.61 ± 43.00
1-Butanol	25.67	56,41,43,39	27.91 ± 28.90^{a}	12.80 ± 5.70^{b}	21.46 ± 11.61^{ab}	15.50 ± 7.80^{ab}	24.11 ± 18.60
1-Pentanol	30.47	55,70,57	1.00 ± 0.26^{a}	0.58 ± 0.09^{b}	0.85 ± 0.32^{a}	0.89 ± 0.24^{a}	0.88 ± 0.25
1-Hexanol	35.77	56,55,43,69	2.37 ± 2.12^{a}	1.21 ± 0.63^{b}	1.85 ± 1.17^{ab}	1.09 ± 0.56^{b}	1.90 ± 1.60
2-Butoxy-ethanol	38.68	57,45,41,87	3.88 ± 5.60^{a}	3.01 ± 3.29^{a}	1.58 ± 1.63^{a}	2.51 ± 1.80^{a}	3.10 ± 4.00
1-Heptanol	40.55	70,56,55,69	0.32 ± 0.06^{a}	0.26 ± 0.06^{b}	0.18 ± 0.08^{c}	0.16 ± 0.05^{c}	0.25 ± 0.08
1-Octanol	43.27	56,69,84,55	0.22 ± 0.07^{a}	0.17 ± 0.03^{b}	0.18 ± 0.06^{ab}	0.16 ± 0.06^{b}	0.18 ± 0.05
2-Alkanols (sum except 2-butanol)			33.50 ± 19.55^{a}	22.87 ± 8.59^{a}	25.01 ± 9.91^{a}	25.5 ± 12.37^{a}	29.95 ± 13.89
2-Propanol	12.78	45,43	25.00 ± 11.41^{a}	18.41 ± 6.78^{a}	18.88 ± 4.83^{a}	21.55 ± 10.51^{a}	23.11 ± 6.41
2-Butanol	19.00	45,59,43,41	126.8 ± 135.0^{b}	487.1 ± 327.0^{a}	496.0 ± 474.0^{a}	215.0 ± 305.0^{b}	141.5 ± 210.0
2-Pentanol	24.44	45,55,73	6.43 ± 6.20^{a}	3.69 ± 1.40^{a}	5.31 ± 4.70^{a}	3.17 ± 1.57^{a}	5.35 ± 5.70
2-Hexanol	28.11	45,69,56,41	0.13 ± 0.05^{b}	0.14 ± 0.09^{b}	0.14 ± 0.05^{b}	0.22 ± 0.13^{a}	0.13 ± 0.09
2-Heptanol	33.74	45,55,83,70	1.73 ± 1.76^{a}	0.60 ± 0.30^{b}	0.67 ± 0.32^{b}	0.52 ± 0.14^{c}	1.30 ± 1.60
2-Nonanol	42.34	45,69,55,70	0.16 ± 0.13^{a}	0.03 ± 0.02^{b}	0.01 ± 0.01^{b}	0.04 ± 0.02^{b}	0.06 ± 0.09
Branched chain alcohols							
2-Methyl-2-Propanol	11.00	59,41,57,42	0.67 ± 0.47^{a}	0.70 ± 0.40^{a}	0.71 ± 0.58^{a}	0.76 ± 0.44^{a}	0.67 ± 0.40
2-Methyl-1-propanol	23.05	43,41,42,33	19.21 ± 12.00^{a}	24.23 ± 19.00^{a}	16.99 ± 12.00^{a}	15.85 ± 6.30^{a}	20.60 ± 17.40
3 Methyl-1-butanol	28.60	55,70,42,57	147.8 ± 92.0^{a}	191.21 ± 106.0^{a}	126.0 ± 88.0^{a}	139.4 ± 68.0^{a}	152.0 ± 106.0
2,6 Dimethyl-heptanol	41.58	69,43,57,87	0.31 ± 0.25^{a}	0.22 ± 0.33^{ab}	0.12 ± 0.18^{b}	0.02 ± 0.06^{c}	0.21 ± 0.30
Unsaturated alcohols			48.25 ± 48.95^{a}	43.12 ± 34.11^{a}	25.15 ± 19.48^{ab}	9.43 ± 7.94^{b}	36.09 ± 47.55
2-Propen-1-ol	24.20	57,39,58,40	46.57 ± 48.50^{a}	41.38 ± 33.50^{a}	23.72 ± 19.00^{ab}	8.09 ± 7.30^{b}	34.51 ± 47.00
1-Penten-3-ol	26.38	57,41,67,71	0.12 ± 0.07^{ab}	0.15 ± 0.12^{a}	0.10 ± 0.10^{ab}	0.05 ± 0.02^{b}	0.10 ± 0.08
2-Buten-1-ol	29.11	57,72,39,53	0.34 ± 0.11^{a}	0.16 ± 0.12^{b}	0.24 ± 0.10^{b}	0.21 ± 0.21^{b}	0.26 ± 0.14
2 Methyl-3-buten-1-ol	30.55	41,56,68,86	0.59 ± 0.09^{b}	0.79 ± 0.18^{a}	0.59 ± 0.14^{b}	0.64 ± 0.14^{b}	0.62 ± 0.13
3 Methyl-3-buten-1-ol	34.06	71,86,41,53	0.63 ± 0.18^{a}	0.64 ± 0.19^{a}	0.50 ± 0.14^{ab}	0.44 ± 0.27^{b}	0.60 ± 0.20

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

of yeasts are found in La Serena cheese [10]. Ethanol has a limited role in the aroma of cheeses despite its high levels, but it contributes to the formation of esters. Higher concentrations of 2-butanol were found in summer and autumn cheeses, although the seasonal period had no significant effect on the level of other major alcohols such as 1-propanol, 2-propanol, 2-pentanol, 2-methyl-1-propanol and 3 methyl-1-butanol. Primary alcohols from 1-butanol to 1-octanol, unsaturated alcohols like 2-propen-1-ol and 2-buten-1-ol, and the secondary alcohols 2-heptanol and 2-nonanol were significantly ($P < 0.05$) more abundant in spring cheeses. Intermediate levels of most alcohols, and lower concentrations of 2-butanol were observed in the HSC. Branched-chain alcohols 2-methyl-1-propanol and 3-methyl-1-butanol come from the reduction of branched-chain aldehydes [18], and high concentrations can be found in raw milk cheeses [11]. The production of these compounds could be favoured in La Serena cheese, where proteolysis is very intense due to the thistle cynarases [19].

3.1.3. Esters

The microorganisms involved in ester formation seem to be mainly yeasts [18], but some lactic acid bacteria and *Micrococcaceae*, together with chemical reactions, can also be responsible [13]. Relatively high amounts of yeasts have been found in La Serena cheese [10]. A high variability between dairies was observed in the ester content of this variety, which is reflected in the high standard deviations (Tab. III). Differences between dairies for 17 out of 23 species of esters were highly significant ($P < 0.001$, data not shown). When seasonal differences in the concentrations of esters were found, the highest levels generally corresponded to spring or summer cheeses. Concentrations of most ethyl esters and branched-chain alkyl esters in the HSC were higher than the mean value for the four seasons. However, levels of propyl esters and ethyl esters of branched-chain acids were lower in the HSC.

3.1.4. Terpenes, hydrocarbons and other compounds

The highest concentration and diversity of terpenes (Tab. IV) were observed in cheeses made during spring and, at a distance, in those made during winter. Limonene, α-pinene, endo borneol, cymene and eucalyptol were the only terpenes detected in cheeses from all seasons. α-Pinene and δ-carene were the most abundant species. Medium concentrations of most terpenes were recorded for HSC. The majority of terpenes found in La Serena cheeses have been detected in the volatile fraction of alpine pastures [16]. They have also been detected in the forages used for cattle feeding, as well as in Saint-Nectaire cheese, and their use as a parameter to control the quality of this cheese variety has been suggested [20]. Terpenes in the volatile fraction of La Serena cheese could have a feed origin, but they may also come from the use of thistle extract as coagulant. In Extremadura Merino sheep graze on natural grass from January to June, until the extremely dry and hot climate conditions dry the grass off. Pastures and feed depend on the season, but also the volatile composition of thistle flowers may vary during storage.

Among the hydrocarbons, n-heptane and n-octane reached maximum levels in summer cheeses. Similar results were reported for Manchego cheese [12]. n-Hexane, 3-methyl-1-heptene, 3,7-dimethyl-2-octene and 1,3-octadiene were significantly ($P < 0.05$) more abundant in spring cheeses. These compounds could also have a feed origin. High concentrations of hydrocarbons were recorded for HSC. Five out of 10 terpenes and 5 out of 7 hydrocarbons were significantly ($P < 0.01$) different between dairies (data not shown).

Table III. Relative abundance (mean ± SD) of esters detected in the volatile fraction of 60-day-old La Serena cheese made throughout the year.

Esters	RT	QI	Spring n =16	Summer n =16	Autumn n =16	Winter n =16	HSC n =10
Ethyl esters							
Ethyl acetate	10.54	43,61,70,88	28.92 ± 10.64 ^{a}	20.69 ± 8.78 ^{b}	24.27 ± 6.79 ^{ab}	17.38 ± 5.78 ^{b}	25.51 ± 9.00
Ethyl propanoate	15.02	57,75,102,74	0.79 ± 0.55 ^{a}	0.51 ± 0.41 ^{b}	0.59 ± 0.26 ^{b}	0.37 ± 0.22 ^{b}	0.51 ± 0.40
Ethyl butanoate	19.99	59,42,60,41	46.41 ± 34.00 ^{a}	24.90 ± 10.10 ^{b}	36.80 ± 23 ^{ab}	29.60 ± 11.00 ^{ab}	43.80 ± 29.00
Ethyl pentanoate	25.28	88,85,57,60	0.34 ± 0.26 ^{a}	0.18 ± 0.08 ^{b}	0.33 ± 0.25 ^{ab}	0.27 ± 0.09 ^{ab}	0.28 ± 0.17
Ethyl hexanoate	29.95	88,99,43,60	23.81 ± 23.11 ^{a}	17.00 ± 7.50 ^{a}	22.40 ± 12.60 ^{a}	22.90 ± 6.40 ^{a}	25.60 ± 17.00
Ethyl heptanoate	34.96	88,43,101,113	0.15 ± 0.17 ^{a}	0.13 ± 0.1 ^{a}	0.19 ± 0.11 ^{a}	0.20 ± 0.11 ^{a}	0.15 ± 0.10
Ethyl lactate	35.57	45,75,103	3.57 ± 1.15 ^{b}	5.01 ± 2.52 ^{a}	3.60 ± 1.13 ^{b}	1.87 ± 0.64 ^{c}	3.89 ± 1.90
Ethyl octanoate	40.02	88,101,127,70	4.51 ± 4.42 ^{a}	3.39 ± 2.59 ^{a}	3.39 ± 2.92 ^{a}	4.11 ± 2.28 ^{a}	4.12 ± 3.50
Ethyl nonanoate	42.84	88,101,141,73	0.04 ± 0.05 ^{a}	0.02 ± 0.03 ^{ab}	0.01 ± 0.01 ^{b}	0.01 ± 0.02 ^{b}	0.02 ± 0.02
Ethyl decanoate	44.84	88,101,157,73	0.14 ± 0.16 ^{a}	0.10 ± 0.07 ^{a}	0.09 ± 0.05 ^{a}	0.11 ± 0.07 ^{a}	0.11 ± 0.10
Branched chain esters							
Ethyl 2 methyl- propanoate	15.53	43,71,88,116	0.15 ± 0.06 ^{a}	0.13 ± 0.05 ^{ab}	0.17 ± 0.14 ^{a}	0.06 ± 0.06 ^{b}	0.08 ± 0.04
Ethyl 3-methyl butanoate	21.70	88,85,57,60	0.21 ± 0.17 ^{a}	0.33 ± 0.14 ^{a}	0.27 ± 0.28 ^{a}	0.18 ± 0.15 ^{a}	0.21 ± 0.15
1-Methyl-propyl acetate	16.88	87,73,61,101	0.02 ± 0.02 ^{b}	0.11 ± 0.10 ^{a}	0.09 ± 0.09^{a}	0.01 ± 0.01^{b}	0.03 ± 0.06
2- Methyl propyl acetate	18.48	43,56,73,41	0.35 ± 0.24 ^{a}	0.17 ± 0.10 ^{b}	0.19 ± 0.10 ^{b}	0.25 ± 0.17 ^{ab}	0.32 ± 0.20
3-Methyl-1-butyl acetate	24.64	43,70,55,87	6.58 ± 5.14 ^{a}	8.25 ± 7.21 ^{a}	4.24 ± 3.84 ^{a}	7.45 ± 7.64 ^{a}	8.60 ± 8.40
3-Methyl-1-butyl-butanoate	31.40	71,70,89,55	0.32 ± 0.34 ^{ab}	0.52 ± 0.45 ^{a}	0.21 ± 0.22 ^{b}	0.39 ± 0.38 ^{ab}	0.39 ± 0.40
Other esters							
Methyl acetate	8.13	43,74,59	0.40 ± 0.19 ^{ab}	0.26 ± 0.14 ^{b}	0.10 ± 0.03 ^{c}	0.43 ± 0.39 ^{a}	0.26 ± 0.18
Propyl acetate	16.16	43,61,73,59	0.28 ± 0.26^{a}	0.33 ± 0.19^{a}	0.35 ± 0.38^{a}	0.16 ± 0.20^{a}	0.24 ± .0.25
Butyl acetate	21.97	43,56,73,61	0.37 ± 0.34 ^{a}	0.16 ± 0.09 ^{b}	0.24 ± 0.11 ^{ab}	0.12 ± 0.04 ^{b}	0.27 ± 0.18
Butyl butanoate	29.30	71,56,89	0.06 ± 0.08 ^{a}	0.01 ± 0.01 ^{b}	0.01 ± 0.02 ^{b}	0.02 ± 0.02 ^{b}	0.04 ± 0.05
1-Methoxy-2-propyl acetate	29.48	43,72,58,87	0.32 ± 0.16 ^{a}	0.20 ± 0.06 ^{b}	0.16 ± 0.06 ^{b}	0.16 ± 0.03 ^{b}	0.22 ± 0.10
Hexyl acetate	31.66	43,56,61,69	0.07 ± 0.08 ^{a}	0.04 ± 0.02 ^{b}	0.04 ± 0.02 ^{b}	0.04 ± 0.03 ^{b}	0.06 ± 0.05
Propyl hexanoate	34.14	99,117,61	0.04 ± 0.07 ^{a}	0.02 ± 0.02 ^{a}	0.02 ± 0.03 ^{a}	0.05 ± 0.04 ^{a}	0.01 ± 0.01

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

Table IV. Relative abundance (mean ± SD) of terpenes and hydrocarbons detected in the volatile fraction of 60-day-old La Serena cheese throughout the year.

Volatile compound	RT	QI	Spring n = 16	Summer n = 16	Autumn n = 16	Winter n = 16	HSC n = 10
Terpenes							
α-Pinene	18.83	93,77,121,91	8.38 ± 8.31 ^{a}	0.08 ± 0.03 ^{b}	0.15 ± 0.16 ^{b}	2.90 ± 6.16 ^{b}	3.84 ± 7.05
Fenchene	20.99	93,79,121,136	0.13 ± 0.14 ^{a}	0.01 ± 0.07 ^{b}	0.01 ± 0.02 ^{b}	0.04 ± 0.06 ^{b}	0.04 ± 0.05
Camphene	21.40	93,121,79,136	0.43 ± 0.46 ^{a}	0.00 ± 0.03 ^{b}	0.01 ± 0.03 ^{b}	0.39 ± 0.83 ^{a}	0.21 ± 0.40
β-Pinene	23.63	93,69,41	0.20 ± 0.17 ^{a}	ND	0.00 ± 0.01 ^{b}	0.06 ± 0.09 ^{b}	0.10 ± 0.10
Thujol	25.46	109,91,67,138	0.14 ± 0.17 ^{a}	0.00 ± 0.01 ^{b}	ND	0.08 ± 0.20 ^{ab}	0.06 ± 0.10
δ-Carene	25.93	93,77,121,136	1.21 ± 2.92 ^{a}	ND	0.00 ± 0.01 ^{b}	0.78 ± 1.38 ^{ab}	0.16 ± 0.30
Limonene	28.47	68,93,121,136	0.33 ± 0.28 ^{a}	0.06 ± 0.08 ^{b}	0.06 ± 0.09 ^{b}	0.04 ± 0.03 ^{b}	0.16 ± 0.30
Eucalipthol	28.98	81,107,139	0.03 ± 0.02 ^{a}	0.01 ± 0.00 ^{b}	0.01 ± 0.01 ^{b}	0.02 ± 0.02 ^{a}	0.02 ± 0.02
Cymene	31.99	119,134,91,115	0.54 ± 0.58 ^{a}	0.06 ± 0.06 ^{b}	0.01 ± 0.01 ^{b}	0.19 ± 0.37 ^{b}	0.20 ± 0.20
Endo borneol	46.00	95,110,139,71	0.09 ± 0.01 ^{a}	0.05 ± 0.00 ^{b}	0.06 ± 0.01 ^{b}	0.05 ± 0.02 ^{b}	0.07 ± 0.02
Hydrocarbons							
n-Pentane	4.03	43,41,57,72	0.28 ± 0.19 ^{a}	0.16 ± 0.06 ^{a}	0.23 ± 0.08 ^{a}	0.24 ± 0.07 ^{a}	0.24 ± 0.09
n-Hexane	4.42	57,43,41,86	1.43 ± 0.61 ^{a}	0.59 ± 0.19 ^{b}	0.43 ± 0.17 ^{b}	0.31 ± 0.21 ^{b}	0.80 ± 0.50
n-Heptane	5.20	43,71,57,100	0.38 ± 0.09 ^{b}	0.54 ± 0.30 ^{a}	0.26 ± 0.08 ^{bc}	0.13 ± 0.04 ^{c}	0.41 ± 0.25
n-Octane	6.97	43,85,57,41	1.56 ± 0.29 ^{b}	2.86 ± 0.96 ^{a}	0.83 ± 0.75 ^{c}	0.54 ± 0.25 ^{c}	1.96 ± 1.03
3-Methyl-1-heptene	8.31	55,41,70,83	0.32 ± 0.16 ^{a}	0.08 ± 0.04 ^{b}	0.18 ± 0.10 ^{b}	0.08 ± 0.33 ^{c}	0.26 ± 0.20
3,7-Dimethyl-2-octene	18.30	70,55,41,140	0.46 ± 0.48 ^{a}	0.05 ± 0.05 ^{b}	0.02 ± 0.02 ^{b}	0.06 ± 0.15 ^{b}	1.42 ± 3.05
1,3-Octadiene	19.50	41,55,67,82	0.08 ± 0.78 ^{a}	0.00 ± 0.01 ^{b}	0.01 ± 0.01 ^{b}	0.02 ± 0.32 ^{b}	0.35 ± 0.70

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

Table V. Relative abundance (mean ± SD) of free fatty acids and aromatic, sulphur and nitrogen compounds detected in the volatile fraction of 60-day-old La Serena cheeses made throughout the year.

Volatile compound	RT	QI	Spring n =16	Summer n =16	Autumn n =16	Winter n =16	HSC n =10
Aromatic compounds							
Toluene	20.31	91,92,65	4.09 ± 2.18 [a]	3.29 ± 3.67 [ab]	1.41 ± 0.78 [b]	1.64 ± 0.67 [b]	2.68 ± 2.41
Ethyl-benzene	25.00	91,106	0.15 ± 0.11 [a]	0.06 ± 0.02 [b]	0.05 ± 0.01 [b]	0.08 ± 0.04 [b]	0.08 ± 0.03
Ethyl benzoate	45.64	105,77,122,150	0.02 ± 0.02 [a]	0.01 ± 0.00 [b]	0.02 ± 0.01 [a]	0.02 ± 0.01 [a]	0.02 ± 0.01
Benzene methanol	46.61	121,43,77	0.24 ± 0.09 [a]	0.18 ± 0.06 [a]	0.14 ± 0.06 [a]	0.22 ± 0.31 [a]	0.20 ± 0.20
Naphthalene	46.91	128,127,129	0.17 ± 0.05 [a]	0.10 ± 0.03 [b]	0.10 ± 0.03 [b]	0.11 ± 0.04 [b]	0.13 ± 0.05
Ethyl 1,2-benzene carboxylate	48.85	90,177	0.02 ± 0.02 [a]	0.02 ± 0.01 [a]	0.01 ± 0.01 [b]	0.01 ± 0.01 [b]	0.02 ± 0.02
Phenol	49.5	94,66	0.11 ± 0.01 [a]	0.05 ± 0.01 [b]	0.04 ± 0.01 [c]	0.03 ± 0.00 [d]	0.05 ± 0.01
Phenylacetaldehyde	45.34	91,92,65,120	0.15 ± 0.01 [a]	0.15 ± 0.03 [a]	0.11 ± 0.02 [b]	0.09 ± 0.03 [c]	0.13 ± 0.04
Acetophenone	45.5	105,77,120,51	0.13 ± 0.01 [a]	0.12 ± 0.01 [a]	0.12 ± 0.02 [a]	0.10 ± 0.01 [b]	0.12 ± 0.01
Sulphur compounds							
Carbon disulphide	5.85	76,78,77	0.07 ± 0.05 [b]	1.71 ± 1.40 [a]	0.58 ± 0.50 [b]	0.09 ± 0.08 [b]	0.66 ± 1.17
Dimethyl sulphide	6.20	62,47,45,35	0.65 ± 0.77 [b]	0.01 ± 0.01 [c]	0.33 ± 0.14 [bc]	1.14 ± 0.99 [a]	0.35 ± 0.36
Dimethyl disulphide	22.20	94,79,45,61	0.49 ± 0.16 [b]	0.24 ± 0.05 [b]	0.21 ± 0.10 [b]	1.13 ± 1.42 [a]	0.32 ± 0.16
Nitrogen compounds							
Acetonitrile	18.18	41,40,39,38	1.49 ± 0.84 [a]	0.64 ± 0.14 [b]	0.65 ± 0.16 [b]	0.84 ± 0.32 [b]	0.88 ± 0.05
Pyrrol	42.69	67,39	0.33 ± 0.05 [a]	0.26 ± 0.01 [b]	0.23 ± 0.05 [b]	0.18 ± 0.03 [c]	0.27 ± 0.07
Free fatty acids			0.90 ± 0.53 [a]	0.29 ± 0.17 [b]	0.23 ± 0.20 [b]	0.16 ± 0.09 [b]	0.49 ± 0.41
Acetic acid	40.80	60,43,45	0.12 ± 0.06 [a]	0.05 ± 0.03 [b]	0.01 ± 0.02 [c]	0.00 ± 0.02 [c]	0.07 ± 0.07
2-Methyl-propanoic acid	43.61	43,73,88,41	0.24 ± 0.19 [a]	0.05 ± 0.06 [b]	0.05 ± 0.05 [b]	0.01 ± 0.02 [c]	0.09 ± 0.10
Butanoic acid	44.69	60,73,55,42	0.24 ± 0.15 [a]	0.08 ± 0.05 [b]	0.05 ± 0.02 [b]	0.05 ± 0.03 [b]	0.14 ± 0.10
3-Methyl-butanoic acid	45.27	60,87,41,74	0.21 ± 0.07 [a]	0.06 ± 0.01 [b]	0.06 ± 0.04 [b]	0.03 ± 0.02 [b]	0.12 ± 0.10
Hexanoic acid	47.41	60,73,87	0.09 ± 0.06 [a]	0.05 ± 0.02 [a]	0.06 ± 0.07 [a]	0.07 ± 0.02 [a]	0.07 ± 0.04

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

Aromatic compounds such as toluene, naphtalene and phenol, and the free fatty acids were more abundant in spring cheeses (Tab. V). Decomposition of sulphur amino acids during cheese ripening produces volatile sulphur compounds such as hydrogen sulphide and methanethiol. Oxidative reactions can convert the latter to dimethyl disulphide [22]. Carbon disulphide was more abundant in La Serena cheeses made in summer, while dimethyl sulphide and dimethyl disulphide showed higher concentrations in winter cheeses (Tab. V). Higher concentrations of sulphur compounds have also been observed in Parmigiano-Reggiano [21], Idiazábal [5], and Roncal [14] cheeses made in winter.

The two nitrogen compounds quantified (Tab. V) were more abundant in La Serena cheeses made in spring. The origin of acetonitrile is unknown but it could be a contaminant. Traces of indole, a degradation product of tryptophan, were also found.

3.2. Sensory analysis.

Analysis of variance showed significant seasonal differences in odour and aroma quality and odour intensity, but not in aroma intensity (Tab. VI). Cheeses made in spring exhibited the highest scores for odour and aroma quality, although differences were only significant compared with the cheeses made in winter. Winter cheeses had the highest scores for odour intensity. The HSC had intermediate scores for odour intensity and low scores for aroma intensity.

3.3. Discriminant analysis

Most volatile compounds were detected in cheeses made during the whole year, although at different concentrations. The only exception were terpenes, not detected in cheeses made during summer and autumn. In summary, concentrations of hydrocarbons, primary alcohols, secondary alcohols of an uneven number of carbon atoms, terpenes, free fatty acids, nitrogen compounds and aromatic compounds were in general significantly higher during spring. Aldehydes reached the highest levels in spring and summer. Ethanol and ethyl esters were more abundant in spring and autumn while branched-chain alcohols and branched-chain alkyl esters were more abundant in summer cheeses. Cheeses made in winter showed lower concentrations of most volatile compounds, except for dimethyl sulphide and dimethyl disulphide.

To select the compounds most influencing the seasonal variability of the volatile profile of La Serena cheese, a discriminant analysis was carried out. The variables used to build the discriminant functions were

Table VI. Sensory scores, on a 0 to 7 points scale, for the quality and intensity of odour and aroma of 60-day-old La Serena cheeses made throughout the year.

Sensory scores	Spring n =8	Summer n =8	Autumn n =8	Winter n =8	HSC n =10
Odour quality	4.37 ± 0.26 ^{a}	4.10 ± 0.47 ^{a}	4.30 ± 0.41 ^{a}	3.52 ± 1.82 ^{b}	4.62 ± 0.30
Aroma quality	4.29 ± 0.32 ^{a}	3.96 ± 0.70 ^{ab}	3.90 ± 0.55 ^{ab}	3.45 ± 1.63 ^{b}	4.66 ± 0.25
Odour intensity	4.10 ± 0.37 ^{c}	4.46 ± 0.45 ^{b}	4.75 ± 0.56 ^{ab}	5.02 ± 0.47 ^{a}	4.67 ± 0.53
Aroma intensity	4.70 ± 0.52 ^{a}	4.44 ± 0.75 ^{a}	4.58 ± 0.69 ^{a}	4.48 ± 0.97 ^{a}	4.37 ± 0.94

HSC, highest scored cheeses. ^{ab} Means followed by the same letter within the same row are not significantly different ($P > 0.05$).

Table VII. Correlation coefficients between the volatile compounds and discriminant functions.

Volatile compound	Function 1	Function 2	Function 3
Phenol	–0.76655*	0.44426	0.03813
1-Methyl-propyl acetate	0.26652*	0.20196	–0.16962
Free fatty acids	–0.19695*	0.18332	0.04195
Ethyl-2-methyl propanoate	0.11062*	0.02955	0.05286
2-Alcanols	–0.08787*	–0.00970	–0.03847
Branched chain aldehydes	–0.14514	0.37607*	0.28898
n-Nonanal	–0.02184	0.31587*	–0.27319
Unsaturated alcohols	0.15286	0.24153*	0.10106
Ethyl lactate	0.02972	0.23755*	–0.09740
Dimethyl sulphide	–0.06349	–0.12092*	–0.01237
3-Methyl-1-butanal	0.12182	0.11822	0.55839*
Phenylacetaldehyde	–0.13210	0.27628	0.40486*
Methyl disulphide	–0.05762	–0.00682	–0.38661*
Diketones	–0.02898	0.11177	0.32001*
Camphene	0.08894	–0.09524	–0.27788*
2,6-Dimethyl-heptanol	–0.10372	0.08757	0.25609*
Methyl acetate	0.12408	0.08293	0.25284*
n-Propanone	–0.01902	0.15950	0.18851*

* Denotes highest absolute correlation between each compound and any discriminant function.

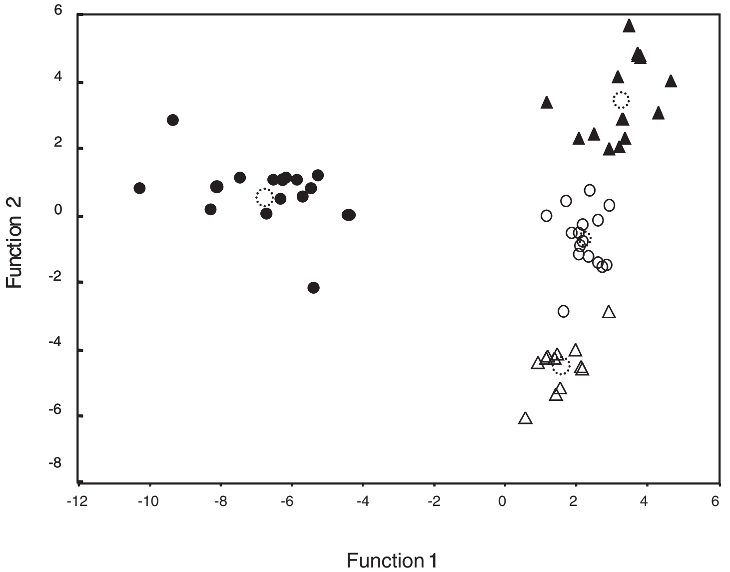

Figure 1. Plot of sample distribution using the two canonical discriminant functions. Cheeses made in spring (●), summer (▲), autumn (○) and winter (△).

those with lower variability between dairies. Table VII lists the correlation coefficients of the variables with the discriminant functions. All the cheeses included in the experiment were classified correctly for the season of manufacture, with the only exception being one cheese made in autumn, which was classified with the winter cheeses (Fig. 1). Function 1 determined the separation of cheeses made in spring. Phenol, free fatty acids and secondary alcohols, more abundant in spring cheeses, correlated negatively with discriminant function 1, whereas 1-methyl-propyl acetate and ethyl-2-methyl propanoate, with lower concentrations in spring cheeses, showed a positive correlation coefficient. Function 2 discriminated cheeses made in summer, autumn and winter. Branched chain aldehydes, more abundant in summer cheeses, n-nonanal, unsaturated alcohols and ethyl lactate correlated positively with function 2, while dimethyl disulphide, more abundant in winter cheeses, correlated negatively. Previous studies have shown that the use of vegetal rennet in the manufacture of La Serena cheese might result in high levels of *Enterobacteriaceae* in curd and cheese [19]. These microorganisms, along with the wild lactic acid bacteria and the thistle extract itself, may contribute to the development of La Serena cheese's aroma and could cause the seasonal variability and differences between dairies in the concentration of some volatile compounds.

The volatile fraction composition of La Serena cheese presents a high variation due to both the season of production and the dairy. The volatile fraction is very rich in alcohols and esters, such as those of other raw milk cheeses, and has the peculiarity of its high diversity of terpenes, especially in spring cheeses. Cheeses made in spring received the highest scores for odour and aroma quality and cheeses made in winter the lowest, coinciding with significantly higher contents of sulphur compounds. Cheeses of lower quality seemed to contain higher amounts of compounds like 2-butanone and 3-methyl-1-butanal, related to the wild microbiota metabolism, and cheeses of higher quality seemed to have lower concentrations of those compounds and higher concentrations of esters, branched-chain aldehydes other than 3-methyl-1-butanal, and free fatty acids.

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