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Characterisation of volatile flavour compounds in Roncal cheese extracted by the 'purge and trap' method and analysed by GC–MS

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Abstract

The objective of this study was to identify the volatile compounds characteristic of Roncal Protected Denomination of Origin (AOC) cheese. Samples were taken from all the cheese makers registered with the Roncal AOC and were compared in the different periods of lactation. The equipment used was a 'purge and trap' system coupled to a gas chromatograph operating with a mass-selective detector. A total of 68 compounds of the following chemical families were detected: hydrocarbons, fatty acids, esters, sulphur and carbonyl compounds and (especially) alcohols. Most of the volatile compounds extracted with the purge and trap appeared in all seasons, but at different concentrations. This allowed a discriminate analysis to classify the samples by the season in which they were made.

1. Introduction

The Roncal Protected Denomination of Origin (AOC) cheese is made in Northeast Navarra (North of Spain) between the months of December and July. It is uncooked pressed cheese made with raw ewes' milk, which must mature at least 4 months before marketing.

The Roncal AOC Regulatory Board function is to protect, promote and authenticate Roncal cheese. To aid in this task, scientific assesment is needed to explain the peculiarities of this product, especially those linked to its origin and traditional methods of preparation. This aims to defend its authenticity against possible imitations as well as to assist new legislative requirements that do not always take the peculiarities of traditional products, as Roncal cheese, into account.

Cheese flavour is the result of the breakdown of milk protein, fat, lactose and citrate due to enzymes from milk, rennet and microorganisms. The interactions of breakdown products, mediated either by enzymes or chemical actions, also play a part. Amino acids and peptides, contributing directly to cheese flavour and aroma, are released during cheese ripening. Certain FAAs are extremely important factors in flavour development, e.g. arginine is related to bitter flavours in cheese, while proline, serine and asparagine are related to sweet flavours (Izco, Torre & Barcina, 2000). Also, the catabolism of amino acids, such as methionine or cysteine, generates sulphur compounds whose presence is a necessary condition for Cheddar cheese flavour (Urbach, 1993).

Free fatty acids (FFAs) form during ripening and are precursors for methylketones, alkanes, and aromatic and aliphatic esters, all of which are characteristic aroma components in cheeses. Moreover, short chain fatty acids contribute directly to aroma in many aged cheeses. Methylketones and related secondary alcohols originating from free fatty acids are the predominant components of blue cheese aroma; methylketones accounted for 75% of the odorous profile of Roquefort cheese while pentan-2-one, heptan-2-one and nonan-2-one were the most abundant ketones. The abundance of FFAs and methylketones could explain the characteristic pungent flavour of the Roquefort cheese (Gallois & Langlois, 1990). The catabolism of lactose and citrate releases diacetyl, acetoin and butan-2,3-diol which can be reduced to butan-2-one and then to butan-2-ol. This group of compounds is certainly important for the characteristic aroma of Cheddar cheese (Urbach, 1993) and fresh cheeses (Fernández-García, 1996). Most of the 70 compounds found in Manchego cheese made with ewe's-milk were short chain FFAs,

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methyketones and ethyl and methyl esters (Martínez-Castro, Sanz, Amigo, Ramos & Martín, Alvarez, 1991). The ethyl and methyl esters are formed in the cheese by esterification of FFAs from lipolysis with ethanol or methanol, respectively, and are responsible for fruity aromas in cheese. Many of them, e.g. ethylbutanoate, ethylhexanoate or ethylheptanoate have been isolated from Cheddar (Urbach), Emmental (Bosset, Gauch, Mariaca & Klein, 1995), Feta (Horwood, Lloyd & Stark, 1981), Parmigiano-Reggiano (Careri, Spagnoli, Panini, Zannoni & Barberi, 1996), and Idiazabal cheeses (Carbonel, 1998), as well as other European cheeses, such as Mahón, Comptè, Beaufort and Appenzeller (Bosset & Gauch, 1993).

There is no ideal technique for extracting and concentrating the volatile compounds that make up the volatile fraction of cheese. Each method researched presents advantages and drawbacks (Bosset & Gauch, 1993). Depending on the method used, the extraction of some compounds will be favoured above others (Bosset et al., 1995). The Purge and Trap technique is applicable for both solid and liquid samples. It is used as a routine technique and for research since it requires small samples (5–25 g), does not need organic solvents and extraction and concentration are performed in one step with low detection limits (Bosset et al., 1995).

The objective of this study was to identify the volatile compounds characteristic of Roncal cheese throughout the entire preparation period. This study is part of broader research subsidised by the Instituto Nacional de Investigaciones Agroalimentarias (INIA, National Institute for Agri-Food Research), the objective of which is to characterise the odour and the aroma of AOC ewe's milk cheese.

2. Materials and methods

2.1. Cheese samples

The cheeses were made in different cheese factories registered with the AOC using the method allowed by the Roncal AOC Regulatory Board (BOE no. 63, 1991). Rennet was added to the ewe's milk at 32°C and allowed to act at that temperature for 45–60 min. The curd so produced was comminuted at a temperature of 38°C. Later, the curds were pressed and moulded. Later still, the cheeses were taken out of the moulds and were brined in a saturated salt solution at 11°C for 18 h. Then they were left to mature under conditions controlled for humidity and temperature for 4 months before they were taken for analysis. The cheeses were transported to the laboratory at 4–6°C in portable coolers. Then, the samples were vacuum-packed and frozen at −80°C until analysis. In this way, all of the samples were analysed under the same conditions over a small period of time.

In each of three preparation seasons (winter, spring and summer) duplicate samples of cheese matured during 4 months were taken from each of the five cheese makers registered with the Roncal AOC. So 10 Roncal cheeses were analysed twice in each of the seasons, which makes a total of 30 cheeses throughout the campaign.

2.2. Sample preparation and purge conditions

Ten grams of finely grated cheese were mixed with 10 g of anhydrous sodium sulphate and placed in an adequate U vial (needle sparger).

The volatile compounds were extracted with an automatic Purge and Trap Sample Concentrator 4460A system (O.I. Analytical, College Station, TX, USA) with a Tenax (O.I. Analytical) trap. The samples placed in the vial were purged under the following conditions: 40ml/min flow of ultra pure helium was used as the purge gas, purge was 20 min at 40°C controlled by a thermal sleeve. The compounds concentrated in the non-cold trap were desorbed at 220°C for 2 min; the valves were kept at 180°C and the transfer line at 120°C. After the desorption, the trap was washed by purging at 220°C for 40 min.

2.3. Chromatographic conditions

The Purge and Trap Sample Concentrator 4460A was connected to an HP 6890 Series GC System coupled to a 5973 Mass Selective Detector (Hewlett Packard, Palo Alto, CA, USA). The data were recorded and analysed with the Hewlett-Packard Enhanced ChemStation G1701AA installed in a Vectra XM 5/166 PC.

The separation of volatile compounds was performed on a HP INNOWax capillary column (60 m×0.25 mm inside diameter) coated with cross-linked polyethylene glycol (0.25 μm film thickness). The separation conditions were as follows: carrier gas was helium at a constant flow of 1 ml/min; split ratio of 30:1; injection port at 200°C. Oven temperature programming was as follows: 32°C for 7 min, then a temperature increase at a rate of 6°C/min up 220°C, and finally 220°C for 5 min (bake). The transfer line (from gas chromatograph to mass spectrometer) was held at 220°C. The detector operated in scan (3 scans/s) mode (total ion Chromatogram) from 19 to 250 amu; ionisation was by electronic ionisation at 70 eV. Ion source was held at 220°C, quadruple at 108°C and calibration was by auto-tuning.

Peak identification was based upon MS spectra comparison with the HP Wiley 275 library and with spectra of injected standards and also on retention times of standards when available.

2.4. Statistical treatment

The SPSS computer program (version 6.1, SPSS Inc., Chicago, IL, USA), was used for statistical processing.

An analysis of variance with 95% confidence intervals was run on the volatile compounds analysed to ascertain whether or not the differences between seasons were significant. Two cheeses from each dairy (10 cheeses)/each season×2 duplicates implies 20 determinations each season. However, not all of the volatile compounds were detected in all of the samples. The analysis of variance was made on volatile compounds detected and quantified at least in six cheeses/each season to be able to compare the averages and establish whether or not the differences were significant. This is reflected in the results displayed in the tables with letters. Different letters (a,b,c) in the same row indicate significant differences between seasons for the same compound (a, higher quantity; ...; c, lower quantity); the same letter means no significant differences.

Stepwise discriminate analysis was also performed on the volatile compounds to ascertain which of the different attributes were most useful in differentiating between seasons and in classifying the cheeses according to manufacturing time. The only variables used were those that were detected and quantified in all the samples (10 cheeses/each season×2 duplicates×3 seasons; $n=60$). Wilk's lambda (λ) was used as the statistical selection criterion for the variables (volatile compounds). When the discriminant functions had been derived, the standardized coefficients in the functions were calculated. The standardized coefficient values plus the correlation values between each component and the discriminant functions were indicative of the relative importance of each of the original attributes (volatile compound) in each of the discriminant functions.

3. Results and discussion

Fig. 1 shows a typical total ion chromatogram of the Roncal cheese volatile fraction extracted by the 'purge and trap' technique. The volatile compounds found in the 4-month-ripened Roncal cheese were the following: five saturated and four unsaturated hydrocarbons, including three aromatic compounds (Table 1); eight primary and five secondary alcohols (Table 2); five aldehydes (Table 3); eight methyl-ketones, one ethyl ketone, and one diketone (Table 4); six acids (Table 5); six esters (Table 6); three sulphur-containing compounds (Table 7); three chlorinated contaminants, hexamethyl cyclotrisiloxane and acetonitrile (Table 8). Also, other compounds were found only in some samples: 3-methylbutanoic acid, ethyl ester (ethyl isovalerate); tetradecane; benzaldehyde; 3-methylbutan-1-ol acetate (isoamylacetate); undecan-2-one; and three chromatographically unresolved peaks (Table 9), corresponding to substances which should be added to the those listed in the previous tables. This all adds up to 68 identified compounds. Most of these compounds had

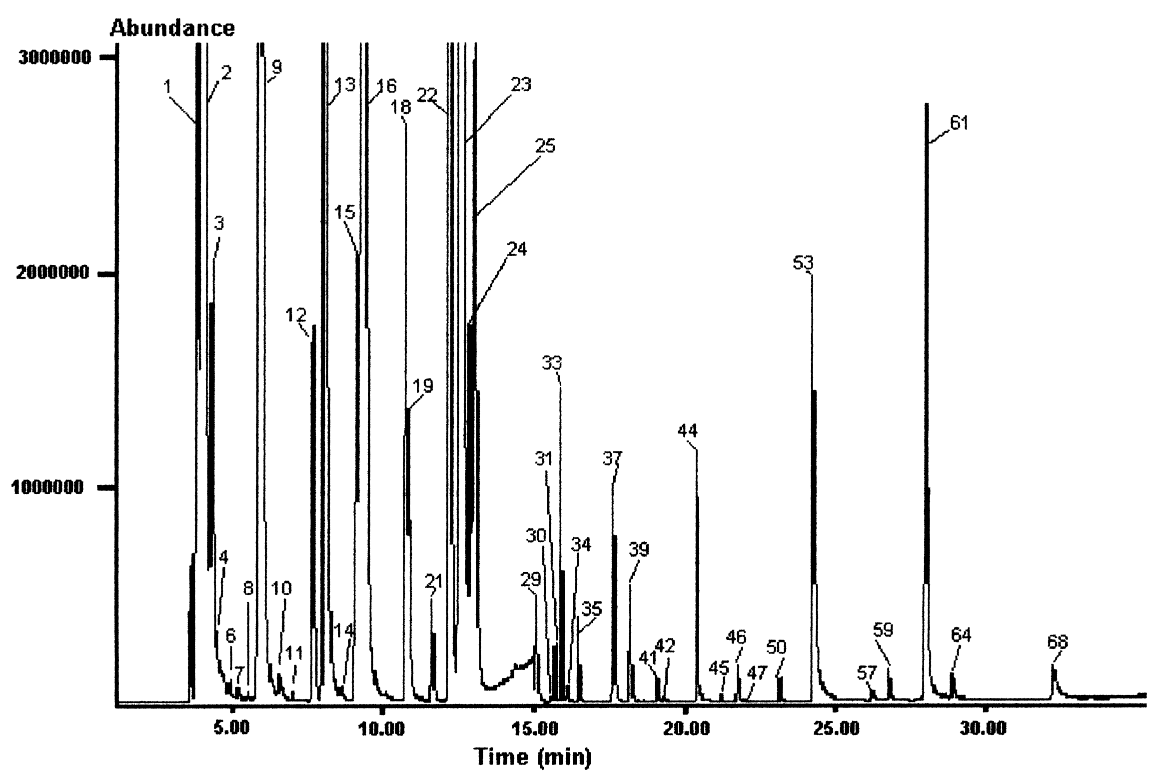

Fig. 1. Total ion chromatogram corresponding to the purge and trap fraction of a 120-days-old Roncal cheese. See Section 2 for experimental details.

Table 1
Hydrocarbons in Roncal cheese (at 120 days of ripening) at three manufacturing times during the lactation periods[a,b]

Peak no.	Hydrocarbons	Winter		Spring		Summer	
		X	RSD	*X*	RSD	*X*	RSD
1	Pentane	4834b	39	6863a	36	6409a	30
2	Hexane	26 949b	18	30 663a	18	32 880a	14
3	Methylcyclopentane (T)	1338b	56	2186a	48	2144a	36
4	Heptane	246b	45	282ab	22	303a	26
7	Methylcyclohexane (T)	96b	60	133a	34	137a	48
10	Octen-2-ene (T)	140c	24	229a	36	187b	21
30	Ethylbenzene*[c]	–	–	17[b]	40	45a	44
32	?-Xylene*[c]	21b	24	43a	51	29	21
43	Styrene*[c]	–	–	23	18	21	32

[a] Arbitrary units (peak area) were used to calculate quantities of each volatile compound. *X*, mean value of 20 determinations (10 cheeses/each season ×2 duplicates). RSD, residual standard deviation in %.

[b] Analysis of variance ($P < 0.05$); different letters in the same row indicate significant differences between seasons (see Section 2.4 for details).

[c] An asterisk denotes compounds not detected in all of the samples: – not detected in any sample. All compounds were confirmed by comparison of retention times and mass spectra of authentic substances except: (T) — tentatively identified, according to Wiley library and to bibliography cited in the References.

Table 2
Alcohols in Roncal cheese (at 120 days of ripening) at three manufacturing times during the lactation periods[a]

Peak no.	Alcohols	Winter		Spring		Summer	
		X	RSD	*X*	RSD	*X*	RSD
16	Ethanol	17 137a	46	15 418a	67	14 345a	56
23	Butan-2-ol	12 597b	84	10 292b	59	29 852a	53
25	Propan-1-ol	1777b	68	1769b	92	3439a	57
29	2-Methylpropan-1-ol	39b	92	110a	110	93ab	89
31	Prop-2-en-1-ol (T)	830a	183	384ab	178	120b	79
33	Pentan-2-ol	199b	119	307a	38	692a	62
34	1-Methoxy-2-propanol* (T)	61a	77	80a	52	84	11
35	Butan-1-ol	85b	43	134b	100	227a	63
39	3-Methylbutan-1-ol	275a	103	627a	133	529a	91
40	Hexan-2-ol*	22b	35	31a	42	25ab	26
42	3-Methylbut-3-en-1-ol	25a	17	25a	24	19b	20
45	Heptan-2-ol	34b	56	60a	44	79a	54
47	Hexan-1-ol*	22a	50	17a	37	20a	40

[a] See footnotes to Table 1.

been found before in other types of cheeses (Bosset et al., 1995; Fernández-García, 1996; Gallois & Langlois, 1990). Even the contaminants (Table 8) and the unresolved peaks (Table 9), had also been found this way (Bosset & Gauch, 1993). Perhaps, the presence of contaminants is due to the fact that they were introduced in the laboratory or it was caused by carry-over problems, which could explain the large quantities of chloroform.

The predominant group was the alcohols, which comprised 19% of the compounds found in the 4-month-ripened Roncal cheese. The most abundant alcohols (taking into account the peak areas) were ethanol, butan-2-ol and propan-1-ol, which have also been detected in large quantities in other cheeses, such as Cheddar (Barlow, Lloyd, Ramshaw, Miller, McCabe & McCabe, 1989) and Feta (Horwood et al., 1981). The secondary alcohols appear as a result of the reduction of the methylketones by the flora bacteria reductases as a defence mechanism of the micro-organisms against toxicity (Molimard & Spinnler, 1996). The primary alcohols, such as ethanol (besides being a direct product of lactose fermentation), 2-methylpropan-1-ol, or 3-methylbutan-1-ol, can be derived from the reduction of the aldehydes formed via Strecker degradation from the amino acids: alanine, valine, and leucine, respectively (Jollivet, Chateaud, Vayssier Bensoussan & Belin, 1994).

The most abundant aldehyde was 3-methylbutanal (Table 3) but, given the low perception threshold of aldehydes [0.07 ppm for pentanal, 0.001–0.004 ppm for nonanal (grass), 0.0018–0.35 ppm benzaldehyde (fresh almonds)] (Moio, Langlois, Etievant & Addeo, 1993), the rest of the aldehydes found can also play a very important role in Roncal cheese. They appear in

Table 3
Aldehydes in Roncal cheese (at 120 days of ripening) at three manufacturing times during the lactation periods[a]

Peak no.	Aldehydes	Winter		Spring		Summer	
		X	RSD	*X*	RSD	*X*	RSD
14	3-Methylbutanal	365a	41	242b	60	274b	37
51	Nonanal*	18a	84	13	62	19a	67
58	Nonenal* (T)	68	102	–	–	–	–
63	Decenal* (T)	31	76	26	60	–	–
66	Undecenal* (T)	19	44	–	–	–	–

[a] See footnotes to Table 1.

Table 4
Ketones in Roncal cheese (at 120 days of ripening) at three manufacturing times during the lactation periods[a]

Peak no.	Ketones	Winter		Spring		Summer	
		X	RSD	*X*	RSD	*X*	RSD
9	Propan-2-one	18 497a	18	19 242a	18	19 781a	10
13	Butan-2-one	26 254a	34	14 912b	42	17 343b	20
18	Pentan-2-one	1221b	59	2149a	44	1446b	56
19	Butan-2, 3-dione	840a	85	992a	52	787a	47
26	Hexan-3-one* (T)	–	–	24	87	–	–
28	Hexan-2-one	21b	42	39a	52	26b	41
44	3-Hydroxybutan-2-one	2275a	119	1652ab	102	689b	64
37	Heptan-2-one	282b	73	1036a	61	800a	62
38	5-Methylhexan-2-one* (T)	21	9	41a	44	46a	57
50	Nonan-2-one	49b	111	310a	81	259a	81

[a] See footnotes to Table 1.

Table 5
Acids in Roncal cheese (at 120 days of ripening) at three manufacturing times during the lactation periods[a]

Peak no.	Acids	Winter		Spring		Summer	
		X	RSD	*X*	RSD	*X*	RSD
53	Acetic acid	459a	157	602a	117	465a	96
57	Propanoic acid*	28a	68	28a	51	31a	45
59	2-Methylpropanoic acid	68a	69	87a	39	78a	57
61	Butanoic acid	593a	82	722a	75	556a	77
64	3-Methylbutanoic acid	102a	74	85a	59	87a	61
67	Hexanoic acid	75a	95	98a	87	70a	90

[a] See footnotes to Table 1.

Camembert cheese after the first week, but quickly transform into the corresponding alcohol or acid (Dumont, Roger & Adda, 1976).

The second most abundant group was the ketones (15% of the total volatile compounds). These compounds, together with the aldehydes, are considered those most responsible for the aroma of blue cheeses. The concentration of ketones in cheese depends on the amount of fat in the original milk (Banks, Brechany, Christie, Hunter & Muir, 1992). Most of the ketones found in Roncal cheese were methylketones (Table 4). Their appearance in cheese is due mainly to the lipolytic action of the micro-flora in cheese. The free fatty acids arose from the lipolysis and are catabolised to methylketones by the micro-organisms. In cheese made with moulds, 60% of the carbonyl compounds produced by these moulds are methylketones formed from free fatty acids (Law, 1981). Important levels of 3-hydroxybutan-2-one (acetoin) appeared and, given its low perception threshold (0.12 ppm), its effect on the aroma of Roncal cheese should be considered very important. It is produced by reduction of butan-2,3-dione (diacetyl); afterwards the acetoin can be reduced to butan-2,3-diol, and this to butan-2-one and then to butan-2-ol (Urbach, 1993). Nevertheless, they can also be synthesised from pyruvate, from lactose and from citrate, which would depend on the lactic acid bacteria, especially *Lactococcus lactis* ssp *lactis* bio-variety *diacetylactis* (Crow, 1990).

Table 6
Esters in Roncal cheese (at 120 days of ripening) at three manufacturing times during the lactation periods[a]

Peak no.	Esters	Winter		Spring		Summer	
		X	RSD	*X*	RSD	*X*	RSD
12	Acetic acid, ethyl ester	158b	63	411a	87	172b	67
36	Butanoic acid, prop-2-enyl ester* (T)	16	15	14	29	16	0
41	Hexanoic acid, ethyl ester	44a	44	59a	49	58a	83
46	Heptanoic acid, ethyl ester*	–	–	–	–	24	38
52	Octanoic acid, ethyl ester*	8a	37	128a	299	8a	62
62	Decanoic acid, ethyl ester*	11a	48	11a	35	11a	29

[a] See footnotes to Table 1.

Of the acids found, the two most abundant are acetic acid and butanoic acid. The fatty acids with six or more carbons are produced by lipase action on the fat. While butanoic, propanoic and acetic acids may also arise from the fermentation of lactose and lactic acid, the acids from the branch chain, 2-methylpropanoiced (isobutyric) and 3-methylbutanoic (isovaleric) are derived from the metabolism of the amino acids (valine and leucine respectively) (Molimard & Spinnler, 1996). They contribute to aroma by being precursors of other compounds (ketones, esters), although it is well known that all those detected in this cheese contribute ''per se'' to the aroma of the mature cheese (Bosset et al., 1995; Careri et al., 1996; Molimard & Spinnler, 1996).

There is no doubt about the importance of esters (formed by esterification among the free fatty acids and the alcohols) in the aroma of the cheese, since esters, with few carbon atoms, have a perception threshold 10

Table 7
Sulfur compounds in Roncal cheese (at 120 days of ripening) at three manufacturing times during the lactation periods[a]

Peak no.	Sulfur compounds	Winter		Spring		Summer	
		X	RSD	*X*	RSD	*X*	RSD
5	Carbon disulfide*	239b	30	341a	36	238b	30
6	Dimethyl sulfide*	132a	31	122a	37	187a	86
27	Dimethyl disulfide*	31	9	14	15	35	72

[a] See footnotes to Table 1.

Table 8
Contaminants in Roncal cheese (at 120 days of ripening) at three manufacturing times during the lactation periods[a]

Peak no.	Contaminants	Winter		Spring		Summer	
		X	RSD	*X*	RSD	*X*	RSD
11	Hexamethyl Cyclotrisiloxane (T)	55b	42	79a	34	73a	25
20	1,1,1-Trichloroethane* (T)	32	–	42b	35	84a	37
21	Acetonitrile	352b	33	394ab	30	453a	17
22	Chloroform	6376b	30	9305a	18	9677a	13
60	2,2-dichloroethanol* (T)	4	–	56a	94	28a	122

[a] See footnotes to Table 1.

Table 9
Unresolved peaks in Roncal cheese (at 120 days of ripening) at three manufacturing times during the lactation periods[a]

Peak no.	Unresolved peaks	Winter		Spring		Summer	
		X	RSD	*X*	RSD	*X*	RSD
8	Propanal/pentanal (T)	55a	30	43b	27	43b	21
15	Propan-2-ol/dichloromethane	781b	32	697b	47	4320a	90
24	Butanoic acid, ethyl ester/toluene	840c	50	1214b	31	1539a	36

[a] See footnotes to Table 1.

times lower than their alcohol precursors. This is due to synergy effects, as is the case with ethyl acetate, butanoate and hexanoate in Cheddar cheese, which produce excessively fruity aromas (Preininger & Grosch, 1994). Like Manchego cheese (Martínez-Castro et al., 1991), the predominant esters in Roncal cheese were the ethyl esters (Table 6).

The sulphur compounds that appear in cheese arise from the catabolism of amino acids and are considered indispensable for achieving the characteristic aromas of cheeses such as Cheddar, Emmental, Camembert and Gruyère. They come mainly from methionine and its later transformation into methanethiol. The sulphur compounds found in Roncal cheese (Table 7) have been detected previously in other cheeses (Fernández-García, 1996). In Domiati cheese, they appear with maturation and, in a greater concentration, in cheese with improper maturation (Collin, Osman, Delcambre, El Zayat & Dufour, 1993). They usually confer a garlic or a very ripe cheese aroma (Jollivet et al., 1994). Since they appeared in only some samples and others mentioned in the literature were not detected, their repercussion in the aroma of Roncal cheese can be assumed to be of little importance.

3.1. Comparison of lactation seasons

Considering the differences among the seasons during which the cheeses were sampled, it seems that most of the compounds are present in all the seasons, but at different concentrations (evaluated by the area of the peaks). It can be concluded that there are differences in the amounts of the volatile compounds, depending on the lactation season. This fact coincides with the volatile fraction studies of Idiazábal ewes' milk cheese (Carbonel, 1998). The results obtained (Tables 1–9) show that cheeses made in winter present lower levels of many of the volatile compounds although they present greater concentrations of some methylketones.

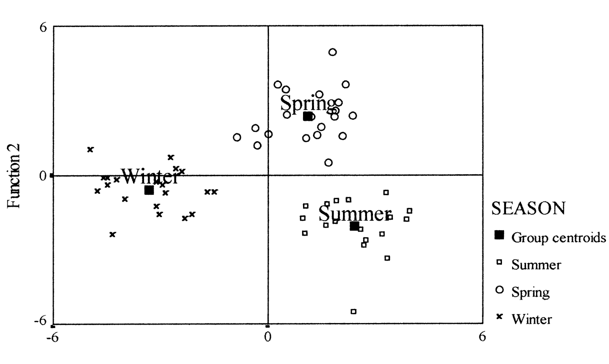

Fig. 2. Plot of sample distribution using the two canonical discriminate functions for the 35 compounds quantified in the 30 duplicate samples analysed ($n=60$).

As can be seen in Fig. 2 there is a clear separation between cheeses made in winter as opposed to those prepared in spring or summer. This classification is determined by function 1, for which the variables indicated in Table 10 are more important. Since the sheep's food affects the raw milk used (Macedo et al., 1996), the differences found might be due to the feeding regime of the sheep (they are kept in stables in winter and fed forage and then taken out to pasture on fresh grass in spring and summer). This could influence the raw material, and consequently the characteristics of the cheese.

Table 10 shows that the first volatile compounds, with greater correlation values, are the methylketone: pentan-2-one, heptan-2-one and their alcohols pentan-2-ol and heptan-2-ol. These methylketones are considered to be quality indices in Cheddar cheese, and especially

Table 10
Pooled within-group correlations between discriminating compounds and canonical discriminant functions (attributes ordered by within-function correlation value)

Volatile compound	Function 1	Function 2
Pentan-2-one	0.26607[a]	0.18344
Pentan-2-ol	0.26145[a]	−0.17575
Heptan-2-ol	0.23942[a]	−0.07291
Heptan-2-one	0.23409[a]	0.18208
Methylcyclopentane	0.23225[a]	0.07011
Hexane	0.23050[a]	0.02177
3-Hydroxybutan-2-one	−0.21663[a]	0.03661
Butan-1-ol	0.19693[a]	−0.12029
Pentane	0.18935[a]	0.00788
Propanal/Pentanal	−0.17524[a]	−0.05309
Butan-2-one	−0.17336[a]	−0.11162
Propan-1-ol	0.14633[a]	−0.10005
2-Methylpropan-1-ol	0.13318[a]	0.08329
Methylcyclohexane	0.13198[a]	0.02485
Prop-2-en-1-ol	−0.11932[a]	0.02490
Ethanol	0.10613[a]	0.07899
3-Methylbutan-1-ol	0.09255[a]	0.06600
Butan-2,3-dione	−0.08742[a]	−0.06076
Heptane	0.05759[a]	0.01347
Propan-2-one	0.05566[a]	0.02542
Butanoic acid	0.05320[a]	0.04486
3-Methylbutanoic acid	−0.02846[a]	0.01196
Butan-2-ol	0.17743	−0.33719[a]
Nonan-2-one	0.15761	0.28552[a]
Acetic acid, ethyl ester	0.07862	0.26195[a]
Hexan-2-one	0.13053	0.24905[a]
Propan-2-ol	0.18903	−0.20821[a]
Hexanoic acid, ethyl ester	0.05945	0.20394[a]
Butanoic acid, ethyl ester/ Toluene	0.14181	−0.19854[a]
3-Methylbutanal	−0.13753	−0.18540[a]
3-Methylbut-3-en-1-ol	0.02681	0.09599[a]
2-Methylpropanoic acid	0.08247	0.08346[a]
Hexanoic acid	−0.03410	0.06286[a]
Acetic acid	0.02189	0.04587[a]
Oct-2-ene	0.02020	0.03931[a]

[a] Denotes largest absolute correlation between each compound and any discriminant function.

pentan-2-one, which can be used as an index of ripening in pressed cheeses with long ripening times, while butan-2-one (with higher concentration values in cheeses made in winter) is related to defects in this cheese (Manning, 1979). Likewise, the above -mentioned alcohols were found necessary to achieve the characteristic Cammembert cheese flavour (Dumont et al., 1976). Pentan-2-one, heptan-2-one, and nonan-2-one (highly correlated with function 1) provide fruity and mouldy flavours, which are characteristics of Blue cheeses as well as Swiss, Gruyère and Camembert cheeses (Carbonel, 1998). The last mentioned author found that the concentrations of heptan-2-ol and nonan-2-one in Idiazabal cheese (very similar to Roncal cheese) made in winter were higher than in spring or summer,while methylketones levels were lower.

Likewise, there is a difference between the cheese made in spring and that in summer or winter, due mainly to the compounds that most correlate with function 2 (Table 10). They include most of the alcohols. In fact, it is in Table 2 (alcohols) where more differences appear between spring and summer cheeses. There are 11 compounds with significant differences between spring and summer cheeses, seven of which are higher in summer (letter "a"). This may be due to greater microbial activity related to the greater presence of micro-flora in the raw milk during the summer months (Mendía, 1998). This may have generated a greater concentration of volatile compound precursors.

As shown in Table 2, one of these alcohols was butan-2-ol (with high correlation value with function 2, Table 10) which had the highest concentration in summer. Butan-2-ol is produced by reduction of butan-2-one; afterwards, the butan-2,3-dione (diacetyl) can be reduced to 3-hydroxybutan-2-one (acetoin), and this to butan-2,3-diol and then to butan-2-one. According to Urbach (1993), production of diacetyl and acetoin (not all the acetoin present in cheese arises from the reduction of diacetyl) and its reduction to butan-2,3-diol can be produced by starter bacteria; however, ulterior reduction to butan-2-one, and this to butan-2-ol is due to adventitious bacteria (those which are there accidentally). Perhaps, a greater presence or activity of adventitious bacteria could explain the highest levels of butan-2-ol found in Roncal cheeses made in summer.

Concentrations of acetic acid, ethyl ester were higher in cheeses made in spring (Table 6), while levels of butanoic acid ethyl ester/toluene were higher in spring or summer than those found in cheeses prepared in winter (Table 9). These compounds are formed by esterification of free fatty acids, originating from lipolysis, with ethanol and are always associated with fruity odour. A possible explanation for our results could be that a greater microbial activity in cheeses made in these seasons has generated a greater lipolysis and therefore a greater concentration of free fatty acids which could esterify with ethanol.

Free fatty acids arose only from the lipolysis as hexanoic acid; those generated also from the catabolism of lactose as butanoic, propanoic or acetic acid, and some acids produced by the breakdown of amino acids such as 2-methylpropanoic acid and 3-methylbutanoic acid, were poorly correlated with the two discriminant functions (Table 10). In fact, no significant differences were found between them (Table 5). Nevertheless, this could be due to the analytical technique used, since according to Fernández-García (1996), quantification of free fatty acids using this analytical column is not very reliable.

The cheeses with odour and flavour shared more characteristics with those made in spring (unpublished data). Therefore, possibly some of the volatile compounds found with higher concentrations in cheeses manufactured in this season have contributed positively to the Roncal cheese aroma, e.g. pentan-2-one or acetic acid, ethyl ester. In general, it seems that the flavour of these cheeses depends, not only on any particular key components, but rather on a 'critical balance' or a 'weighted concentration ratio' of all components present. Nevertheless, this does not imply that some of the components are not more important than others.

It must be pointed out that the differences found among the seasons should be interpreted with caution, since the RSD values are high (Tables 1–9). There is heterogeneity (within the same season) among the cheese factories registered with the Roncal AOC with reference to the levels of many of the volatile compounds. The heterogeneity of the raw material (as to physical-chemical values and micro-biological counts) pointed out on other occasions (Arizcun, Barcina & Torre, 1997), may have caused this.

4. Conclusion

The compounds that make up the aroma of Roncal cheese belong to the following families: hydrocarbons, fatty acids, esters, sulphur- and carbonyl compounds, and especially alcohols.

The study of the differences among the lactation periods of winter, spring and summer shows that most of the volatile compounds extracted with the 'purge and trap' technique appeared in all the seasons, albeit with different concentrations. In terms of these results, it can only be speculated that the flavour of these cheeses seems to depend, not only on particular key components, but rather on a 'critical balance' or a 'weighted concentration ratio' of all components present. Nevertheless, this does not imply that some of the components are less important than others, for example pentan-2-one or acetic acid, ethyl ester. This could explain the higher odour and aroma scores obtained for the cheeses made in spring.

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References

Arizcun, C., Barcina, Y., & Torre, P. (1997). Identification of lactic acid bacteria isolated from Roncal and Idiazábal cheese. *Le Lait, 77*, 723–730.

Banks, J. M., Brechany, E. Y., Christie, W. W., Hunter, E. A., & Muir, D. D. (1992). Volatile components in steam distillates of Cheddar cheese as indicator indices of cheese maturity, flavour and odour. *Food Research International, 2*, 365–373.

Barlow, I., Lloyd, G. T., Ramshaw, E. H., Miller, A. J., McCabe, G. P., & McCabe, L. (1989). Correlations and changes in flavors and chemical parameters of Cheddar cheese during maduration. *Australian Journal of Dairy Technology*, *44*, 7–18.

BOE. *Boletín Oficial del Estado* (1991, March 14). No. 63. *Regulatory Board of the Roncal cheese apellation of origin*. Spain: Ministerio de Agricultura, Pesca y Alimentación.

Bosset, J. O., & Gauch, R. (1993). Comparison of the volatile flavour compounds of six European AOC cheeses by using a new dynamic headspace GC–MS method. *International Dairy Journal, 3*, 359–377.

Bosset, J. O., Gauch, R., Mariaca, R., & Klein, B. (1995). Comparison of various sample treatments for the analysis of volatile compounds by GC–MS: application to the Swiss Emmental cheese. *Mitteilungen-aus-derm-gebiete-dem-lebensmitteluntersuchung-und-hygieen, 86*, 672–698.

Carbonel, M. (1998). *Characterisation of the Idiazábal cheese volatile fraction. Effect of starters and of the pasteurisation of the milk*. PhD thesis, Universidad Pública de Navarra, Spain.

Careri, M., Spagnoli, S., Panini, G., Zannoni, M., & Barberi, G. (1996). Chemical parameters of non-volatile fraction of ripened Parmigiano-Reggiano cheese. *International Dairy Journal, 6*, 147–155.

Collin, S., Osman, M., Delcambre, S., El Zayat, A. Y., & Dufour, J. P. (1993). Investigation of volatile flavor compounds in fresh and ripened Domiati cheeses. *Journal of Agricultural and Food Chemistry, 41*, 1659–1663.

Crow, V. L. (1990). Properties of the 2,3-butanediol dehydrogenase from *Lactococcus lactis* sbsp. *lactis* in relation to citrate fermentation. *Applied Environmental Microbiology, 56*, 1656.

Dumont, J. P., Roger, S., & Adda, J. (1976). L'arome du Camembert: autres composès mineurs mis en èvidence. *Le Lait, 56*, 595–599.

Fernández-García, E. (1996). Use of Headspace Sampling in the Quantitative Analysis of Artisanal Spanish Cheese Aroma. *Journal of Agricultural and Food Chemistry, 44*, 1833–1839.

Gallois, A., & Langlois, D. (1990). Volatile compounds of French blue cheeses. *Le Lait, 70*, 89–106.

Horwood, J. F., Lloyd, G. T., & Stark, W. (1981). Some flavour components of feta cheese. *Australian Journal of Dairy Technology, 36*, 34–37.

Izco, J. M., Torre, P., & Barcina, Y. (2000). Ripening of Ossau-Iraty cheese: determination of free amino acids by RP–HPLC and of total free amino acids by the TNBS method. *Food Control, 11*, 7–11.

Jollivet, N., Chateaud, J., Vayssier, Y., Bensoussan, M., & Belin, J. (1994). Production of volatile compounds in model milk and cheese media by eight strains of *Geotrichum candidum* Link. *Journal of Dairy Research, 61*, 241–248.

Law, B. A. (1981). The formation of aroma and flavour compounds in fermented dairy products. *Dairy Science Abstract, 43*, 143–154.

Macedo, A. C., Costa, M. L., & Malcata, F. X. (1996). Assessment of proteolysis and lipolysis in Seroza cheese: effects of axial cheese localitation, ripening time and lactation season. *Le Lait, 76*, 363–370.

Manning, D. J. (1979). Cheddar cheese flavour studies. II Relative flavour contributions of individual volatile components. *Journal of Dairy Research, 46*, 523–559.

Martínez-Castro, I., Sanz, J., Amigo, L., Ramos, M., & Martín Alvarez, P. (1991). Volatile components of Manchego cheese. *Journal of Dairy Research, 58*, 239–246.

Mendía, C. (1998) *Proteolytic and organoleptics changes in Idiazábal cheese throughout all preparation periods: effect of starters and of the pasteurisation of the milk*. PhD thesis. Universidad Pública de Navarra, Spain.

Moio, L., Langlois, D., Etievant, P., & Addeo, F. (1993). Powerful odorants in water buffalo and bovine Mozzarella cheese by use of extract dilution sniffing analysis. *Italian Journal of Food Science, 3*, 227–237.

Molimard, P., & Spinnler, H. E. (1996). Compounds involved in the flavor of surface mold-ripened. *Journal of Dairy Science, 79*, 169–184.

Preininger, M., & Grosch, W. (1994). Evaluation of key odorants of the neutral volatiles of Emmentaler cheese by the calculation of odour activity values. *Lebensmittel-Wissenschaft + Technologie, 27*, 237–244.

Urbach, G. (1993). Relations between cheese flavour and chemical composition. *International Dairy Journal, 3*, 389–422.