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# Comparison of dynamic headspace and simultaneous distillation extraction techniques used for the analysis of the volatile components in three European PDO ewes' milk cheeses

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**Abstract**

This preliminary study aims to compare two different extraction and concentration methods often used for the gas chromatographic analysis of volatile components in food to determine the advantages and drawbacks of both for future routine investigation of PDO (protected designation origin) ewes' milk cheeses. Roncal, Pecorino Sardo and Fiore Sardo were investigated at different ripening stages. The dynamic headspace technique using a Purge & Trap device (DHS) makes it possible to extract more highly volatile compounds than the simultaneous distillation extraction (SDE) method. Consequently, the latter is more efficient for extracting low-volatile components such as phenols, free fatty acids, lactones and longer-chain aldehydes, ketones, alcohols and esters. These two extraction methods are therefore complementary. 

*Keywords:* Volatile component; Dynamic headspace technique; Simultaneous distillation extraction; Ewes' milk cheese

## 1. Introduction

The instrumental characterisation of the volatile fraction is important not only for defining cheese composition, but also for linking it with its typical organoleptic features and original environment, as required for a protected designation origin (PDO). The extraction of volatile compounds from cheese is difficult due to the complexity of the matrix (Urbach, 1993). Various analytical techniques have been proposed (Mariaca & Bosset, 1997; Bosset & Gauch, 1993), each of them having advantages and drawbacks.

Volatile fractions from some ewes' milk cheeses have already been studied (Ortigosa, Torre, & Izco, 2001; Fernández-García, 1996; de Frutos, Sanz, & Martínez-Castro, 1991; Ha & Lindsay, 1991; González de Llano, Ramos, Polo, Sanz, & Martínez-Castro, 1990; Horwood, Lloyd, & Stark, 1981). There have been some attempts to find a relationship between the volatile fraction and the microflora of the cheese as well as to determine the metabolic pathway for the biosynthesis or the biodegradation of some volatile compounds (Ortigosa et al., 2001; Moio, Dekimpe, Etievant, & Addeo, 1993b; Collin, Osman, Decambre, El-Zayat, & Dufour, 1993). However, so far the volatile fractions of Pecorino Sardo and Fiore Sardo (both from Italy) cheeses have not been investigated by dynamic headspace technique using a Purge & Trap device (DHS) or simultaneous distillation extraction (SDE). In the case of Roncal cheese (Spain), the composition of the volatile fraction was recently examined by DHS (Ortigosa et al., 2001) as well as supercritical fluid extraction (SFE) (Larráyoz, Ibáñez, Torre, Ordóñez, & Barcina, 1999).

These types of cheese are made from raw ewes' milk according to the manufacturing process approved by their PDO Regulatory Board (Table 1). Roncal and Pecorino Sardo cheeses are semi-hard-type cheeses; Fiore Sardo is a hard cheese. Some physico-chemical and quality parameters of these varieties have been studied. Composition and evolution of the microflora, proteolysis and lipolysis have also been studied in Roncal cheese (Arizcun, Barcina, & Torre, 1997a, b; Oria Almudia & Sala Trepat, 1992; Millán, Alcalá,

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Table 1
Characteristics of the ewes' milk cheeses studied

| Variety | Age of samples (months) | Milk | Type of cheeses | Starter | Rennet | Brine | Typical ripening duration (days) |
|---|---|---|---|---|---|---|---|
| Roncal | 5 | Raw | Semi-hard, unsmoked | Addition (optional) | Natural lamb's or commercial | Saturated | 120 |
| Pecorino Sardo | 1 | Raw | Semi-hard, unsmoked | Addition of natural culture | Industrial calf rennet | Saturated | 60 |
| Pecorino Sardo | 7 | Raw | Semi-hard, unsmoked | Addition of natural culture | Industrial calf rennet | Saturated | 180 |
| Fiore Sardo | 8 | Raw | Hard, smoked | No starter | Natural lamb or kid paste rennet | Saturated | 180 |

Sanjuán, Penedo, & Castelo, 1992), in Pecorino Sardo cheese (Scintu, Ledda, Floris, & Sanna, 1995; Ledda, Scintu, Pirisi, & Piredda, 1996) as well as in Fiore Sardo cheese (Ledda, Murgia, & Arrizza, 1978; Botazzi, Arrizza, & Ledda, 1978; Pettinau, Nuvoli, & Podda, 1978; Ledda, Scintu, Pirisi, Sanna, & Mannu, 1994; Piredda et al., 1995; Piredda et al., 1996; Pirisi, Pinna, & Papoff, 1999; Pinna, Pirisi, Piredda, Addis, & Di Salvo, 1999).

The aim of this preliminary study was to compare two analytical techniques, a DHS using a Purge & Trap device (Mariaca & Bosset, 1997) and a SDE, both coupled with gas chromatography (GC) with mass selective and flame ionisation detections (MSD + FID) (Godefroot, Sandra, & Verzele, 1981) to determine semi-quantitatively the volatile components (measured as peak height) of three ewes' milk cheeses at different ripening grades ($n = 1$ sample per cheese type and ripening grade). This comparison of methods has to demonstrate their advantages and drawbacks for future routine investigation of PDO ewes' milk cheeses.

## 2. Materials and methods

The comparison of the methods applied in the different laboratories participating in this investigation explains the various types of equipments used.

### 2.1. Samples

Roncal PDO cheese at 5 months of ripening, Pecorino Sardo PDO cheese at 1 and 7 months of ripening and Fiore Sardo PDO cheese at 8 months of ripening were tasted and selected as high-grade material. A representative sample was obtained by pooling two pieces from opposite ends of each cheese block and grating them after discarding 1 cm of rind and smear. The grated sample was stored frozen at −20°C until analysis.

### 2.2. Dynamic headspace

#### 2.2.1. Reagents and chemicals

Authentic compounds used to confirm mass spectra and retention indices are marked with a star (*) in Tables 2–7. The Milli-Q water® was boiled under a continuous nitrogen flow for ≈15 min with an electric heating device to strip off all residual volatile trace components.

#### 2.2.2. Sample preparation

Two different procedures were used: a suspension method and a dry method.

*2.2.2.1. Suspension method.* Shortly before analysis, a representative amount of ≈20 g of the sample material was weighed and finely dispersed in 80 mL water with a high-speed homogeniser (Polytron PT 3000 used with a PT-DA 6030–6060 cutting system, Kinematica) running at 5000 rpm for 5 min. Finally, 20 mL of this mixture was carefully introduced into a 25 mL non-fritted sparger of the Purge & Trap extraction system.

*2.2.2.2. Dry method.* Approximately, 10 g of sample material was weighed and introduced directly into a 25 mL non-fritted sparger of the Purge & Trap extraction system.

#### 2.2.3. Extraction of the volatiles

The Purge & Trap system LSC 2000 (Tekmar, Cincinnati, OH, USA) included a 25 mL non-fritted sparger (Schmidlin Co, art. no.14-2333-4SL, CH-6345 Neuheim), a trap (No. 8, containing a mixture of Carbosieve SIII (0.05 g) and Carbopack B 60/80 (0.2 g)) as well as a cryofocusing unit. The moisture control module was not used. The operating conditions were as follows: purge gas, nitrogen; purge flow (vent), 30 mL $min^{-1}$; prepurge, 1.5 min; water bath, 45°C; purge, 10 min; dry purge, 10 min; cap cool-down, −125°C; desorb preheat to 210°C; desorb, 4 min at 220°C; inject, within 1 min from −125°C to 200°C; bake,

Table 2

(A) *Hydrocarbons and terpenes found using the dynamic headspace technique coupled with GC/MS*[a]

| Peak no. | Compounds | LRI[b] (SPB-1) | Roncal | | Pecorino Sardo | | | | Fiore Sardo | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 5 Months | | 1 Month | | 7 Months | | 8 Months | | |
| | | | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry | |
| 7 | 1,4-Pentadiene | 516 | | | | | | 1,060,000 | | | |
| 9 | 1,3-Pentadiene | 526 | | | | | 78,000 | 33,000 | | | |
| 24 | Benzene* | 655 | | 360 | | 1110 | | | | | 9, 10 |
| 54 | 2,4-Dimethyl-pentane | 816 | | | | | | | 300 | | |
| 51 | 2,4-Dimethyl-hexane | 800 | | | | | | | 700 | | |
| 75 | 2,2,4,6,6-Pentamethyl-heptane | 1032 | | | | | | | 1500 | 860 | |
| 30 | Hept-1-ene* | 690 | 723 | | | | | | | | |
| 41 | Toluene* | 761 | 1970 | 4030 | 1420 | 2750 | 1460 | 2370 | 3100 | 3900 | 9 |
| 47 | Trans1-butyl,2-methyl-cyclopropane | 790 | 1340 | | | | | | | | |
| 49 | Oct-4-ene | 797 | | 3920 | | | | | | | |
| 50 | Oct-3-ene | 800 | 1890 | 992 | | 661 | 1100 | 3760 | | | |
| 52 | Octane* | 803 | | | | 1660 | | | | | 9, 10 |
| 53 | Oct-2-ene* | 811 | | 281 | | | | | | | 10 |
| 68 | 3,7-Dimethyl-1,6-octadiene | 945 | | | | | 918 | 944 | | | |
| 71 | 3,7-Dimethyl-2-octene | 969 | | | | | | 772 | | | |
| 78 | 3-Methyl, 5-propyl nonane | 1052 | | | | | | | 2400 | 1400 | |
| 79 | Nonadecane | 1065 | | | | | | | 439 | | |
| 69 | α-pinene | 947 | 455 | 479 | | | | | 381 | | |
| 76 | Limonene | 1038 | 132 | 358 | | | | | | | 9 |

(B) *Hydrocarbons and terpenes found using simultaneous distillation extraction (SDE) coupled to GC–MS*[a]

| Peak no. | Compounds | LRI[c] (INNOWax) | Roncal | Pecorino Sardo | | Fiore Sardo | References |
|---|---|---|---|---|---|---|---|
| | | | 5 Months | 1 Month | 7 Months | 8 Months | |
| 5 | Toluene | 1036 | 536 | | | | 1, 4, 9 |
| 10 | Benzene 1,3-dimethyl | 1157 | 378 | 222 | 1420 | | 2, 9 |
| 25 | *n*-Tetradecane | 1421 | | | | | 4, 5, 10 |
| 39 | 2,3-Dimethyl, 2-cyclopenten-1-one | 1669 | | | | 3469 | |
| 44 | *n*-Pentadecane | 1691 | 361 | | | | 5 |
| 48 | Heptadecane | 1773 | 457 | | | | 10 |
| 53 | Benzene, 1,2-dimethoxy | 1867 | | | | 422 | |
| 57 | 2-Hexadecene | 1976 | 583 | | | | |

[a] Note: Values indicated peak heights.
[b] LRI = linear retention index using an SPB-1 column.
*MS-Identification confirmed by comparison of Rt's of authentic compound.
(1) Horwood et al. (1981).
(2) Collin et al. (1993).
(3) González de Llano et al. (1990).
(4) Moio et al. (1993b).
(5) de Frutos et al. (1991).
(6) Ha and Lindsay (1991).
(7) Fernández-Garcia (1996).
(8) Moio and Addeo (1998).
(9) Careri, Manini, Spagnoli, Barbieri, and Bolzoni (1994).
(10) Ortigosa, Torre and Izco (2001).
[c] LRI = linear retention index using an HP-INNOWax column.

5 min at 260°C; 6-port valve, 150°C; line, 150°C; capillary union heater (transfer line from purge and trap to gas chromatograph), 150°C.

*2.2.4. Gas chromatography*

Qualitative and semi-quantitative GC analyses were carried out using a Hewlett-Packard (HP) 5890 Series II under the following operating conditions: carrier gas, helium; inlet pressure, 40 kPa; flow, $\approx 1.6\,mL\,min^{-1}$ at 45°C; transfer line (from GC to MS), 280°C; interface, direct inlet; temperature programme, 13 min at 45°C, heating rate, $5°C\,min^{-1}$ to 240°C, and 5 min at 240°C; capillary column, SPB1 (Supelco), 30 m × 0.32 mm i.d., film thickness, 4 μm. Two detectors were mounted in parallel by splitting the flow at the end of the capillary column (split ratio: ≈1:1 at 45°C), i.e. a Hewlett-Packard flame ionisation detector (FID) and a mass-sensitive detector (MSD model HP 5972), operating in the scan mode (TIC) from 19 to 250 amu at $2.9\,scans\,s^{-1}$, ionisation by EI at 70 eV by autotuning; MS-Scan after 3.5 min. The MSD is used for the identification of the volatile (flavour) compounds, the FID for their relative quantification.

*2.3. Simultaneous distillation extraction*

*2.3.1. Reagents and chemicals*

The same reference compounds (*, Tables 2–8) as mentioned above were used. All reagents and chemicals used were obtained from Sigma (St. Louis, MO, USA) or Merck (Munich, Germany). They were of "Purum" grade or better. *N*-pentane was supplied by Panreac (Montcada y Reixat, Spain).

*2.3.2. Sample preparation*

The grated cheese sample was weighed (5 g) to a precision of 1 mg and finely dispersed in 50 mL ultrapure water. Finally, 300 μL of a methanolic camphor solution ($0.035\,mg\,mL^{-1}$) was added as an internal standard and the sample was homogenised using an Ultra-Turrax® (IKA-Labortechnik, Germany).

*2.3.3. Extraction of the volatiles*

A microversion of the distillator–extractor (Chrompack, Middelburg, The Netherlands) was used in a configuration suitable for a solvent lighter than water (Godefroot et al., 1981).

*2.3.4. Gas chromatography*

Semi-quantitative GC analysis was performed using an HP 5870 (Hewlett-Packard, Avondale, PA, USA) equipped with a crosslinked polyethylene glycol column HP-INNOWax (60 m × 0.22 mm i.d. × 0.25 μm film thickness). Helium was employed as carrier gas with a flow of $1\,mL\,min^{-1}$. Injection volume was 1 μL by split mode with a split ratio of 1:60; injector temperature was 200°C. The FID was operated at 250°C. The oven temperature programme was as follows: 35°C for 1 min, $3°C\,min^{-1}$ to 170°C, $4°C\,min^{-1}$ to 200°C, 200°C for 20 min. Chromatograms were recorded using two-level attenuation.

Identification (qualitative analysis) of volatile compounds was performed with a gas chromatograph 6890 HP coupled with a 5973 HP MSD. The analysis was carried out under the following conditions: ionisation voltage, 70 eV; source temperature, 230°C; scanned range, 19–300$m/z$; scanned velocity, $3\,scans\,s^{-1}$. The elution conditions were the same as those detailed above.

## 3. Results and discussion

The qualitative and semi-quantitative results are summarised in Tables 2–8. The components identified are listed by chemical functional groups. Tables A list the volatile compounds found in each cheese ($n = 1$) and with each extraction method (suspension or dry) using the DHS. Tables B show the results obtained using the SDE. The values indicate the peak height (arbitrary units) of each compound. Fig. 1 (on the top: the dry method; on the bottom: the suspension method) and Fig. 2 show the volatile profile from Fiore Sardo cheese by DHS and SDE techniques, respectively.

*3.1. Hydrocarbons*

The DHS (Table 2A) made it possible to detect the hydrocarbons with a low boiling point, particularly using the dry extraction method. On the other hand, the heavier hydrocarbons were easily detected using the SDE technique (Table 2B). The first technique detected methyl benzene (toluene) in all cheese varieties analysed, while the SDE technique detected toluene in the Roncal cheese only. This aromatic hydrocarbon, already detected in the Beaufort and Comté cheese types (Dumont & Adda, 1978; Dumont, Adda, & Rousseaux, 1981), may be due to the freezer storage of the samples (Bosset, Gubler, Bütikofer, & Gauch, 2000).

The Roncal cheese was the only cheese type that contained mono-unsaturated hydrocarbons such as 2-octene, 4-octene and 1-heptene. 3-Octene was found in Roncal and in Pecorino Sardo cheese types. 1,4-Pentandiene, 1,3-pentandiene and 3,7-dimethyl-1,6-octadiene were detected in the 7-month-ripened Pecorino Sardo cheese only.

*3.2. Alcohols*

The DHS (Table 3A), particularly the so-called "dry method", was more efficient in the extraction of light primary alcohols. Among the primary alcohols

Table 3

(A) *Alcohols found using the dynamic headspace technique coupled with GC/MS*[a]

| Peak no. | Compounds | LRI[b] (SPB-1) | Roncal | | Pecorino Sardo | | | | Fiore Sardo | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 5 Months | | 1 Month | | 7 Months | | 8 Months | | |
| | | | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry | |
| 2 | Ethanol* | | 110,000 | 824,000 | 710,000 | 1,190,000 | 18,000 | 191,000 | 9100 | 705,000 | 5, 7, 9, 10 |
| 5 | Propan-2-ol* | | 2860 | 21,300 | | | 1220 | 11,000 | 60,000 | 221,000 | 5,7, 10 |
| 8 | 2-Propen-1-ol | 526 | 2130 | 17,400 | | | | | | 1300 | |
| 11 | Propan-1-ol* | 537 | 7440 | 46,400 | 10,000 | 22,000 | 522 | 1500 | 3900 | 13,000 | 5, 7, 9, 10 |
| 17 | Butan-2-ol* | 586 | 143,000 | 192,000 | 187,000 | 180,000 | 3312 | 4320 | 44,700 | 62,600 | 1, 5, 7, 9, 10 |
| 19 | 2-Methyl-propan-1-ol* | 617 | 2010 | 3960 | 22,000 | 25,000 | 673 | 1500 | 16,000 | 27,500 | 1, 7, 9, 10 |
| 23 | Butan-1-ol* | 653 | 1860 | 6350 | | | 1040 | 2800 | 12,000 | 23,500 | 5, 8, 9, 10 |
| 36 | 3-Methyl-butan-1-ol* | 721 | 8960 | 17,900 | 82,000 | 141,000 | 3420 | 3400 | 49,300 | 52,700 | 7, 8, 9, 10 |
| 37 | 2-Methyl-butan-1-ol* | 725 | 1650 | 29,600 | 9000 | 21,000 | 562 | | 9200 | 10,800 | 7, 8 |
| 40 | Pentan-1-ol* | 752 | | | | | 1330 | 1460 | 1200 | 770 | 3, 5, 6, 7, 8, 9 |
| 29 | Pentan-2-ol* | 687 | 715 | 24,200 | | | 4850 | 12,000 | 97,000 | 123,800 | 7, 8, 9, 10 |
| 57 | Hexan-2-ol* | 852 | | | | | | | 1700 | | 1, 5, 6 |
| 33 | Heptan-1-ol* | 700 | | | | | | 927 | | | 3, 8, 9 |
| 63 | Heptan-2-ol* | 885 | | | | | 1590 | | 1600 | | 3, 7, 8 |

(B) *Alcohols found using the simultaneous distillation extraction (SDE) coupled with GC/MS*[a]

| Peak no. | Compounds | LRI[c] (INNOWax) | Roncal | Pecorino Sardo | | Fiore Sardo | References |
|---|---|---|---|---|---|---|---|
| | | | 5 Months | 1 Month | 7 Months | 8 Months | |
| 4 | Butan-2-ol* | 1016 | | 3263 | | | 2, 7, 9, 10 |
| 9 | Pentan-2-ol | 1118 | 839 | | 1385 | 33,063 | 1, 2, 7, 8, 9, 10 |
| 14 | Butan-1-ol, 3-methyl* | 1214 | 2897 | 12,771 | | 9117 | 5, 9, 10 |
| 15 | Hexan-2-ol* | 1234 | | | | 1357 | |
| 21 | Heptan-2-ol* | 1334 | 2852 | 154 | 2699 | 18,151 | 3, 4, 8, 9 |
| 24 | Octan-2-ol | 1399 | 323 | | | | |
| 30 | Hexan-1-ol, 2-ethyl | 1474 | 123 | | | | 4, 7, 9 |
| 36 | Nonan-2-ol | 1574 | 1043 | | 5175 | 2518 | 5 |
| 38 | Nonen-2-ol | 1636 | 275 | 139 | | | 8 |
| 40 | Endo-borneol* | 1669 | 76 | | | | |
| 63 | Benzene methanol | 2044 | | 1218 | | 7039 | |
| 65 | Benzene ethanol* | 2089 | 278 | 2558 | | | 4 |

[a] See Table 2 footnotes.

Table 4

(A) *Ketones found using the dynamic headspace technique coupled with GC/MS*[a]

| Peak no. | Compounds | LRI[b] (SPB-1) | Roncal | | Pecorino Sardo | | | | Fiore Sardo | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 5 Months | | 1 Month | | 7 Months | | 8 Months | | |
| | | | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry | |
| 4 | Propanone* | | 10,100 | 71,200 | 4700 | | 56,000 | 492,000 | 160,000 | 1,113,000 | 10 |
| 14 | Butan-2-one* | 569 | 227,000 | 429,000 | 161,000 | 370,000 | 7520 | 17,000 | 16,700 | | 1, 5, 6, 7, 9, 10 |
| 13 | Butan-2,3-dione | 558 | | 5490 | 3500 | 70,000 | 790 | 17,000 | | 1700 | 7, 10 |
| 12 | 3-Buten-2-one | 557 | 1440 | | | | | | | | |
| 35 | 3-Penten-2-one | 716 | | | | | | | | 500 | |
| 28 | 3-Hydroxy-butan-2-one | 684 | | | | 6600 | | | | | 6, 8, 9, 10 |
| 25 | Pentan-2-one* | 673 | 5100 | 17,300 | 1400 | 4440 | 313,000 | 563,000 | 427,000 | 636,300 | 1, 3, 5, 7, 9, 10 |
| 38 | 3-Methyl-pentan-2-one* | 738 | | | | | 719 | 1400 | 2100 | 2500 | 8 |
| 44 | Hexan-2-one* | 771 | | | | | 14,000 | 13,000 | 6700 | 11,300 | 3, 6, 8, 9, 10 |
| 61 | Heptan-2-one* | 872 | 978 | 939 | 782 | 780 | 134,000 | 106,000 | 53,000 | 32,400 | 2, 3, 6, 7, 8, 9, 10 |
| 66 | 3-Methyl-heptan-2-one | 930 | | | | | 258 | | | | |
| 72 | Octan-2-one* | 969 | | | | | | 442 | | | 6, 8 |
| 80 | Nonan-2-one* | 1075 | | | | | 16,000 | 3700 | | 1400 | 3, 5, 6, 7, 8, 9, 10 |
| 43 | Cyclopentanone | 766 | | | | | | | | 5900 | |
| 56 | Cyclohexanone | 820 | | | | | | | 400 | | |

(B) *Ketones found using simultaneous distillation extraction (SDE) coupled with GC/MS*[a]

| Peak no. | Compounds | LRI[c] (INNOWax) | Roncal | Pecorino Sardo | | Fiore Sardo | References |
|---|---|---|---|---|---|---|---|
| | | | 5 Months | 1 Month | 7 Months | 8 Months | |
| 2 | Pentan-2-one | 971 | 1003 | 867 | 23,336 | 15,927 | 4, 7, 8, 10 |
| 8 | Hexan-2-one* | 1092 | 142 | 466 | 1285 | 1948 | 1, 3, 4, 6, 8, 10 |
| 12 | Heptan-2-one* | 1199 | 6471 | 1891 | 103,317 | 85,451 | 1, 2, 3, 4, 5, 6, 7, 8, 10 |
| 19 | Octan-2-one | 1319 | 262 | | 2813 | 1501 | 4, 8 |
| 20 | 3-Hydroxy-butan-2-one* | 1331 | | 401 | | | 4, 7, 8, 9, 10 |
| 26 | Nonan-2-one* | 1436 | 17,132 | 1052 | 139,188 | 53,452 | 2, 3, 4, 6, 8, 10 |
| 32 | 8-Nonen-2-one | 1494 | | | 3924 | 835 | 8 |
| 37 | 3,5-Octadien-2-one | 1580 | | 326 | | | |
| 43 | Undecan-2-one | 1680 | | | 11,452 | 1273 | 4, 5, 8 |
| 59 | Dodecan-2-one | 1980 | | 317 | 2304 | | 8 |

[a] See Table 2 footnotes.

Table 5

(A) *Aldehydes found using the dynamic headspace technique coupled with GC/MS*[a]

| Peak no. | Compounds | LRI[b] (SPB-1) | Roncal | | Pecorino Sardo | | | | Fiore Sardo | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 5 Months | | 1 Month | | 7 Months | | 8 Months | | |
| | | | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry | |
| 1 | Ethanal* | | 3010 | 14,400 | 46,000 | 106,000 | 222,000 | 730,000 | 3000 | 20,300 | 1, 7 |
| 3 | 2-Propenal | | 2200 | | | | | | | | |
| 10 | 2-Methyl-propanal* | 531 | | | 860 | | 2200 | 1500 | 800 | 640 | 10 |
| 20 | 2-Butenal | 627 | | | | | 519 | 1700 | | | |
| 21 | 3-Methyl-butanal* | 638 | 484 | 859 | 111,000 | 20,000 | 7200 | 10,000 | 25,700 | 35,400 | 1, 6, 7, 9, 10 |
| 22 | 2-Methyl-butanal* | 649 | | | 16,000 | 3900 | 4600 | 6500 | 6800 | 10,100 | 1, 7, 9, 10 |
| 26 | Pentanal* | 678 | | | | | | | 13,500 | 18,600 | 1, 6, 9 |
| 45 | Hexanal* | 780 | | | | | 595 | 521 | 1500 | | 1, 6, 9 |
| 82 | Nonanal* | 1089 | 2240 | 375 | | | | | | | 8, 9, 10 |
| 85 | Decanal* | 1193 | 3670 | 258 | | 276 | | | | | 8, 10 |

(B) *Aldehydes found using simultaneous distillation extraction (SDE) coupled with GC/MS*[a]

| Peak no. | Compounds | LRI[c] (INNOWax) | Roncal | Pecorino Sardo | | Fiore Sardo | References |
|---|---|---|---|---|---|---|---|
| | | | 5 Months | 1 Month | 7 Months | 8 Months | |
| 1 | 3-Methyl-butanal | 957 | | | | 6870 | 2, 4, 7, 8, 9, 10 |
| 7 | Hexanal | 1089 | 500 | 1347 | | | 9 |
| 13 | 2-Heptanal | 1207 | | 1420 | | | |
| 28 | Nonanal | 1441 | | 359 | | | 4, 8, 9, 10 |
| 34 | Methional* | 1506 | 123 | 270 | 382 | | 8 |
| 35 | 2,4-Heptadienal | 1529 | | 238 | | | |
| 42 | Phenyl acetaldehyde | 1674 | | 459 | | | 4 |
| 41 | 2-Nonenal | 1669 | 275 | 139 | | | |
| 49 | Benzene acetaldehyde | 1775 | | | 2551 | 1172 | |
| 58 | 2,4-Decadienal | 1977 | | 708 | | | |
| 61 | Tetradecanal | 2017 | | | | | |
| 66 | Unknown | 2124 | | | 1225 | | |

[a] See Table 2 footnotes.

Table 6

(A) *Esters found using the dynamic headspace technique coupled with GC/MS*[a]

| Peak no. | Compounds | LRI[b] (SPB-1) | Roncal | | Pecorino Sardo | | | | Fiore Sardo | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 5 Months | | 1 Month | | 7 Months | | 8 Months | | |
| | | | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry | |
| 16 | Formic acid, ethyl ester* | 580 | | | | | | | | 490 | 1, 10 |
| 6 | Acetic acid, methyl ester | 511 | | | | | | | | 950 | |
| 18 | Acetic acid, ethyl ester* | 599 | 3700 | 7700 | 5900 | 11,000 | 2800 | 12,000 | 54,500 | 78,600 | 5, 6, 7, 10 |
| 32 | Acetic acid propyl ester | 698 | 503 | 956 | | | | | | 2100 | 1, 5 |
| 39 | Acetic acid, 1-methyl propyl ester | 746 | 594 | 797 | | | | | | | |
| 48 | Acetic acid, butyl ester | 795 | | | | | | | 770 | | 9 |
| 58 | Acetic acid, 3-methyl butyl ester | 860 | | | | | | | 1900 | 1500 | |
| 31 | Propanoic acid, ethyl ester* | 696 | | | 320 | | | | 1700 | 2400 | 5, 9 |
| 34 | Butanoic acid, methyl ester* | 707 | | 662 | | | 1050 | 1560 | 25,300 | 33,500 | 8, 9 |
| 46 | Butanoic acid, ethyl ester* | 785 | 8930 | 9340 | 12,000 | 13,000 | 2200 | 2900 | 216,000 | 213,700 | 1, 3, 5, 6, 7, 8, 9 |
| 55 | Butanoic acid, isopropyl ester | 816 | | | | | | | 6900 | | 8 |
| 62 | Butanoic acid, propyl ester* | 880 | 492 | | | | | | 8200 | 5600 | 1, 8, 9 |
| 67 | Butanoic acid, 2-methyl propyl ester | 939 | | | | | | | 4400 | 2800 | |
| 59 | Butanoic acid, 2-propenyl ester | 865 | | | | | | | 1000 | | |
| 77 | Butanoic acid, 3-methyl butyl ester | 1039 | | | 1400 | | | | 8200 | 4600 | |
| 65 | Butanoic acid, hexyl ester | 922 | | | | | | | 2500 | | |
| 64 | Hexanoic acid, methyl ester* | 905 | | | | | | 523 | 4800 | 3100 | 3, 8, 9 |
| 73 | Hexanoic acid, ethyl ester* | 979 | 1170 | 778 | 3750 | 2500 | 420 | 201 | 42,800 | 25,000 | 2, 6, 7, 8, 9, 10 |
| 74 | Hexanoic acid, 1-methyl ethyl ester | 1018 | | | | | | | 1500 | 880 | |
| 83 | Hexanoic acid, 2-methyl propyl ester | 1136 | | | | | | | 468 | | |
| 81 | Hexanoic acid, propyl ester* | 1076 | | | | | | | 2700 | | 9 |
| 84 | Octanoic acid, ethyl ester* | 1179 | | | | 602 | | | 1600 | 420 | 3, 2, 6, 7, 8, 9 |
| 86 | Octanoic acid, 3-methyl butyl ester | 1237 | | | | | | | 600 | | |

(B) *Esters found using simultaneous distillation extraction (SDE) coupled with GC/MS*[a]

| Peak no. | Compounds | LRI[c] (INNOWax) | Roncal | Pecorino Sardo | | Fiore Sardo | References |
|---|---|---|---|---|---|---|---|
| | | | 5 Months | 1 Month | 7 Months | 8 Months | |
| 6 | Butanoic acid, ethyl ester* | 1044 | | | | 7264 | 1, 2, 3, 5, 6, 7, 8, 9 |
| 11 | Butanoic acid, 2-methyl propyl ester | 1174 | | | | 510 | |
| 16 | Butanoic acid, butyl ester | 1240 | | | | 693 | 8, 9 |
| 17 | Hexanoic acid, ethyl ester* | 1249 | 454 | 520 | 1585 | 12,278 | 2, 3, 5, 6, 8, 9, 10 |
| 18 | Butanoic acid, methyl propyl ester | 1292 | | | | 4629 | |
| 22 | Acetic acid, heptyl ester | 1386 | 720 | | | | |
| 23 | Hexanoic acid, propyl ester | 1389 | | | | 909 | |
| 27 | Hexanoic acid, 2-methyl propyl ester | 1436 | 159 | | | 941 | |
| 29 | Hexanoic acid, butyl ester | 1455 | | | | 976 | 8 |
| 31 | Octanoic acid, ethyl ester* | 1477 | 301 | | | 5462 | 7, 8, 9 |
| 33 | Hexanoic acid, 3-methyl butyl ester | 1503 | | | | 3115 | |
| 46 | Decanoic acid, methyl ester* | 1705 | 271 | | | 9314 | 2, 3, 5, 9 |
| 50 | Decanoic acid, ethyl ester* | 1791 | | | | 10,740 | 2, 3, 4, 5, 6, 8, 9, 10 |
| 51 | Octanoic acid, isoamyl ester | 1816 | | | | 3056 | |
| 56 | Dodecanoic acid, methyl ester* | 1975 | 40 | | | 1287 | 5, 8 |
| 64 | Dodecanoic acid, ethyl ester* | 2057 | 238 | | | 4550 | 4, 5, 6, 8 |
| 76 | Octadecanoic acid, methyl ester | 2422 | 703 | | | | |
| 78 | 9-Octadecenoic acid, methyl ester | 2466 | 7348 | | | | |
| 79 | 9,12-Octadecenoic acid, methyl ester | 2478 | 384 | | | | |

[a] See Table 2 footnotes.

Table 7

(A) *Fatty acids found using the dynamic headspace technique coupled with GC/MS*[a]

| Peak no. | Compounds | LRI[b] (SPB1) | Roncal | | Pecorino Sardo | | | | Fiore Sardo | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 5 Months | | 1 Month | | 7 Months | | 8 Months | | |
| | | | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry | |
| 15 | Acetic acid* | 570 | | | | | | | | 10,200 | 7, 8, 9, 10 |
| 27 | Propanoic (Propionic) acid* | 684 | | | | | | | | 3500 | 8, 9, 10 |
| 42 | Butanoic (Butyric) acid* | 765 | | | | | | | 2800 | 12,900 | 3, 4, 5, 7, 8, 9, 10 |
| 60 | Pentanoic acid | 869 | | | | | | | | 1600 | 3, 4, 8, 9 |
| 70 | Hexanoic (Caproic) acid* | 951 | | | | | | | | 6000 | 3, 4, 7, 8, 9 |

(B) *Fatty acids found using simultaneous distillation extraction (SDE) coupled with GC/MS*[a]

| Peak no. | Compounds | LRI[c] (INNOWax) | Roncal | Pecorino Sardo | | Fiore Sardo | References |
|---|---|---|---|---|---|---|---|
| | | | 5 Months | 1 Month | 7 Months | 8 Months | |
| 45 | 2-Methyl propanoic acid* | 1691 | | | | 1090 | 6, 8, 10 |
| 47 | Butanoic acid* | 1721 | 1174 | | 1283 | 217,924 | 3, 4, 5, 6, 7, 8, 9, 10 |
| 54 | Pentanoic acid | 1892 | | | | 6973 | 6, 8, 9 |
| 55 | 4-Methyl pentanoic acid | 1953 | | | | 7559 | 6 |
| 60 | Hexanoic acid* | 2001 | 6043 | 794 | 20,544 | 1,653,057 | 3, 4, 6, 7, 8, 9 |
| 62 | 3-Methyl pentanoic acid | 2029 | | | | 4566 | 6 |
| 67 | Heptanoic acid* | 2140 | 210 | | 522 | 19,199 | 4, 6, 7, 8, 9 |
| 72 | Octanoic acid* | 2241 | 17,769 | 537 | 63,867 | 1,370,344 | 2, 3, 4, 5, 6, 8, 9 |
| 74 | Nonanoic acid | 2357 | 349 | | 903 | 17,154 | 6, 8, 9 |
| 77 | Decanoic acid* | 2446 | 26,361 | 4943 | | 1,322,056 | 2, 3, 4, 5, 6, 8 |
| 80 | 9-Decenoic acid | 2519 | 993 | | | 21,972 | 6 |
| 81 | Undecanoic acid | 2561 | 210 | | | 5548 | |
| 83 | Dodecanoic acid* | 2657 | 4212 | 1645 | 14,151 | 87,076 | 2, 3, 5, 8 |
| 84 | Tetradecanoic acid* | 2756 | 1306 | 916 | 11,452 | 15,150 | 2, 3, 5, 8 |

[a] See Table 2 footnotes.

Table 8
Lactones, phenols and miscellaneous found using simultaneous distillation extraction (SDE) coupled with GC/MS[a]

| Peak no. | Compounds | LRI[b] (INNOWax) | Roncal | Pecorino Sardo | | Fiore Sardo | References |
|---|---|---|---|---|---|---|---|
| | | | 5 Months | 1 Month | 7 Months | 8 Months | |
| 52 | δ-Octalactone | 1826 | | | | 1056 | 4, 8 |
| 73 | γ-Decalactone | 2249 | 118 | | | | 4, 8 |
| 75 | δ-Dodecalactone | 2395 | 238 | | 596 | | 4, 5 |
| 82 | Hexadecalactone | 2621 | | | 531 | | 4 |
| 68 | 4-Methyl phenol* | 2162 | 343 | 110 | | 5819 | 4, 6 |
| 69 | 2-Methyl phenol* | 2174 | | | | 5543 | 4 |
| 70 | Phenol-4-methyl-2-methoxy | 2184 | | | | 3521 | 4 |
| 71 | Phenol* | 2209 | | | | 3581 | 4, 9 |
| 3 | Chloroform* | 999 | 2107 | | 700 | 5842 | 9, 10 |

[a] See Table 2 footnotes.

identified (C2–C7) ethanol was the most abundant in all cheese types. It originates from a direct fermentation of acetaldehyde by lactic acid bacteria (Imhof, Isolini, & Bosset, 1990). The formation of other primary alcohols in cheese may be mainly due to the reduction of the correspondent aldehydes (Dumont & Adda, 1979; Behnke, 1980; McSweeney & Sousa, 2000). The SDE technique (Table 3B) detected predominantly secondary alcohols in the range C4–C9.

Methyl-branched primary alcohols, 2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol were detected in all cheese types analysed using the DHS. 3-Methyl-1-butanol, which develops a pleasant aroma of fresh cheese (Moio, Dekimpe, Etievant, & Addeo, 1993a), is very abundant in the unripened Pecorino Sardo cheese. These branched-chain primary alcohols are produced by the reduction of the corresponding aldehydes which are derived from isoleucine, valine and leucine, respectively.

Among the secondary alcohols, heptan-2-ol was found in all cheese varieties. Butan-2-ol was the most abundant in Roncal and 1-month-ripened Pecorino Sardo cheeses. The ripened Pecorino Sardo cheese mainly contains pentan-2-ol and the Fiore Sardo cheese more propan-2-ol. The secondary alcohols, especially those with an odd carbon number, are derived by the reduction of the corresponding methyl ketones. Such components and in particular heptan-2-ol and nonan-2-ol are typical components of blue cheese flavour (de Frutos et al., 1991).

During the ripening of Pecorino Sardo cheese a decrease in the concentration of several alcohols was observed: ethanol, propan-1-ol, butan-2-ol, 2-methyl-1-propanol, 3-methyl-1-butanol and 2-methyl-1-butanol. On the other hand, an increase in the concentration of propan-2-ol, butan-1-ol, pentan-1-ol, pentan-2-ol, heptan-1-ol, heptan-2-ol and nonan-2-ol was found. The strong reducing condition in hard cheeses may favour the formation of alcohols from aldehydes and ketones.

### 3.3. Ketones

The same trend was observed using both of the techniques.

The DHS (Table 4A) and especially the dry method were once again more efficient for the extraction of ketones in comparison with the SDE (Table 4B) technique, even though the latter is able to extract longer-chain methyl ketones up to C12. All the methyl ketones with an odd number of carbons (C3–C9) were detected in all cheese varieties. They are formed at considerably higher levels than those with an even number of carbons (C4, C6, C8, C12). It is possible, even likely, that the odd-numbered methyl ketones are not present, or only present at a lower concentration in the unheated cheeses and are formed from β-ketoacid glycerides during the heating used in the extraction methods.

With the exception of butan-2-one and propan-2-one, Roncal cheese generally contained lower quantities of methyl ketones than Sardinian cheeses. In Pecorino Sardo cheese, the amount of methyl ketones generally increased during ripening. Undecan-2-one occurred only in very ripened Sardinian cheeses (7-month-old Pecorino Sardo and 8-month-old Fiore Sardo cheeses).

These volatile components are frequently present in many cheese varieties. They were detected in very high amounts in mould-ripened cheeses, where they play a key role because of their characteristic flavour (Behnke, 1980). Methyl ketones are probably derived from β-ketoacid triglycerides with β-keto-acids as intermediate products (Kinsella & Hwang, 1976; Adda, 1986).

Diacetyl, revealed with the DHS only, was found in all cheese types. It was more abundant in unripened

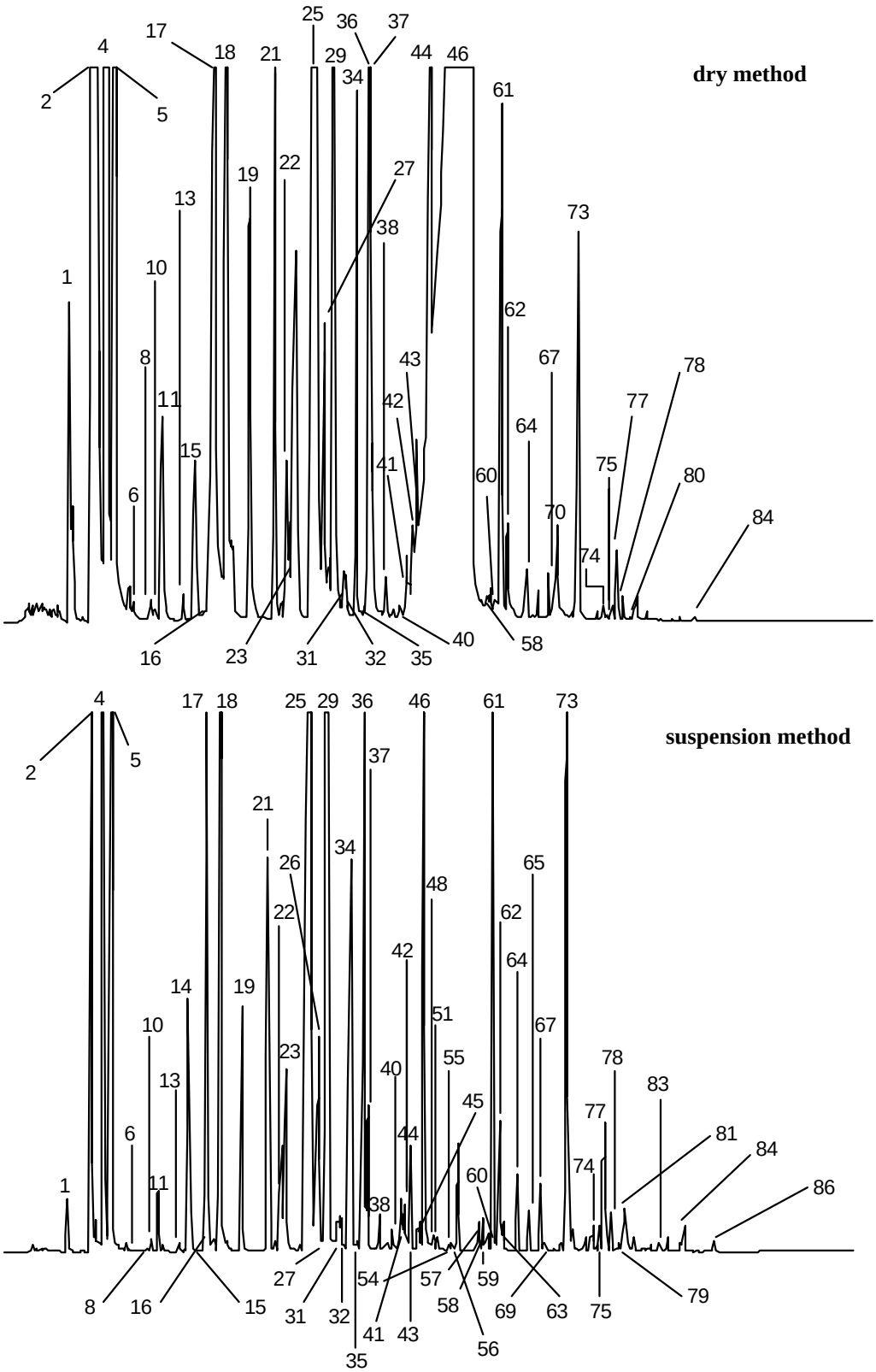


Fig. 1. Gas chromatography profile of volatile compounds extracted by dynamic headspace by dry method (top) and suspension method (bottom) from Fiore Sardo cheese at 8-month ripening. (The peak numbers refer to Tables 2–8.)

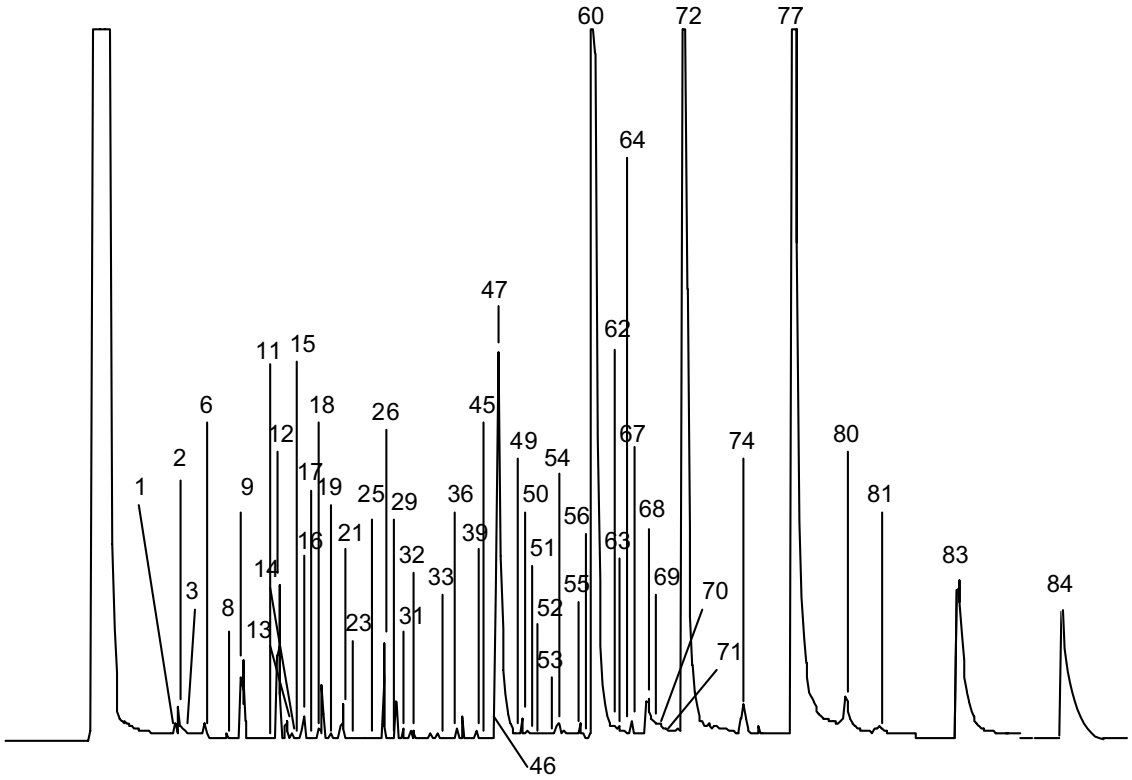


Fig. 2. Gas chromatography profile of volatile compounds extracted by simultaneous distillation extraction (SDE) from Fiore Sardo cheese at 8-month ripening. (The peak numbers refer to Tables 2–8.)

Pecorino Sardo cheese where it decreases during ripening, being converted to acetoin. The former compound has a buttery nut-like flavour (Welsh, Murray, & Williams, 1989). The acetoin/diacetyl couple was detected only in the 1-month-old Pecorino Sardo cheese. These compounds are derived from citrate/lactate metabolism.

### 3.4. Aldehydes

The same trend was established by the efficient extraction of the highly volatile aldehydes using the DHS (Table 5A), although the SDE (Table 5B) technique gives a better extraction for the aldehydes with a higher boiling point.

The DHS showed that acetaldehyde was the most abundant aldehyde in all cheese types except for Fiore Sardo. It increases during Pecorino Sardo ripening; this component is produced during lactose metabolism by lactic acid bacteria, but also by breakdown of threonine (Imhof et al., 1990). Similar to the trend for ketones, aldehydic compounds detected by DHS are more abundant in 7-month-old Pecorino Sardo and 8-month-old Fiore Sardo cheeses. These cheeses contained pentanal which was not found in the unripened Pecorino Sardo and the Roncal cheeses. On the other hand, these latter cheeses contain nonanal and decanal.

The straight-chain aldehydes (C4–C10) are formed during $\beta$-oxidation of unsaturated fatty acids (Collin et al., 1993). The branched-chain 2-methyl propanal, 2-methyl butanal and 3-methyl butanal are present especially in the Sardinian cheeses, and probably originate from amino acid degradation (valine, isoleucine and leucine) by enzymatic pathways, as well as non-enzymatic processes (Behnke, 1980; Griffith & Hammond, 1989). The tentative interpretation of the above-mentioned results is, nevertheless, doubtful due to the possible spontaneous $\beta$-oxidation of the unsaturated fatty acid during the frozen storage of the cheese sample.

### 3.5. Esters

The esters derived from acetic, propionic, butyric, caproic and caprylic acids were detected better by DHS (Table 6A). Using the SDE technique (Table 6B), the medium and longer chain esters, such as ethyl decanoate, dodecanoate, tetradecanoate and octadecanoate, were extracted.

Ethyl acetate, ethyl butyrate and ethyl hexanoate occurred in all cheese types. These substances decreased during the ripening of Pecorino Sardo cheese. Fiore Sardo cheese showed the highest content of esters. In this cheese the most abundant ester was ethyl butyrate, which was found in fruity-tasting cheeses such as

Gruyère and Parmesan; ethyl esters are known for their strong fruity character (Barbieri et al., 1994; Arora, Cornier, & Lee, 1995).

Esterification of fatty acids with the primary alcohols occurs by an enzymatic or a chemical pathway (Behnke, 1980; Dumont & Adda, 1979).

### 3.6. Fatty acids

Fatty acids, with C2–C6 chains, were detected using the DHS (Table 7A) in the dry mode in the Fiore Sardo cheese only. The SDE technique (Table 7B), once again, was able to extract medium- or longer-chain fatty acids. The latter technique in fact made it possible to identify all short- and medium-chain fatty acids (C2–C14) in all cheeses.

In cheese, free fatty acids ($\geqslant$4) originate from the lipolysis of milk fat (Urbach, 1993). Lipolysis may be due to the action of the native milk lipases in cheese made from raw milk, to the action of microbial lipases, even though lactic bacteria present in starter cultures only have a weakly lipolytic activity, or the action of the lipases present in the paste rennet used in cheesemaking. The free fatty acids content of Fiore Sardo cheese could be due to the lipase content of the rennet used for its manufacture. It is well known that the paste rennet used for the Fiore Sardo cheesemaking has high lipase activity (Pinna et al., 1999).

Over the ripening period considered for Pecorino Sardo (1 and 7 months) the free fatty acids increased. With the exception of decanoic and decenoic acids, the Roncal cheese had a lower content of the C4–C14 free fatty acids in comparison with the ripened Pecorino Sardo cheese, but the highest amount of free fatty acid was detected in the Fiore Sardo cheese.

Fatty acids are important components for the flavour of many cheese types. In particular, the short-chain fatty acids impart a desirable piquant taste to Italian cheeses such as Fiore Sardo cheese.

### 3.7. Lactones

The DHS was unable to detect the lactones. On the other hand, the SDE technique (Table 8) detected two $\delta$-lactones (C8, C12) and two $\gamma$-lactones (C10, C12) already described by Urbach (1993). The importance of these compounds as well as esters, responsible for the fruity odour, is due to their low detection threshold, especially $\gamma$-lactones (Dufossé, Latrasse, & Spinnler, 1994). Some authors postulate a biosynthesis based on the dehydration and cyclisation generated from hydroxy fatty acids during ripening (Behnke, 1980; Dumont & Adda, 1979). These substances did not occur in the unripened Pecorino Sardo cheese.

### 3.8. Phenols

Phenolic compounds (Table 8) have very low perception thresholds, 1 ppb or less, with odour descriptors like sweet, medicinal, ewe, smoked, unpleasant. (Urbach, 1997). Such components, particularly those substituted with methyl and ethyl groups, were found in high concentration in the Fiore Sardo cheese only when using the SDE technique. Some authors described a phenolic odour as characteristic of ewes' milk cheese. In fact, this compound occurred at a higher level in ewes' than in cows' milk cheese (Moio et al., 1993b). Phenol is generated from the microbial breakdown of tyrosine (Parliment, Kolor, & Rizzo, 1982) or from conjugates by enzymes or heat.

### 3.9. Other components

Some terpenes such as $\alpha$-pinene in Roncal and Fiore Sardo cheeses and limonene in the Roncal cheese were only detected using the DHS (Table 2A). They most probably originate from the activity of the microorganisms in the cheese (Berger, 1999) and not from the botanical composition of forage, which generally leads to the simultaneous occurrence of $\alpha$- and $\beta$-pinene in the sample. This effect was clearly demonstrated in the highland region (Mariaca et al., 1997; Bosset et al., 1997).

## 4. Conclusions

The DHS technique extracts more highly volatile compounds than SDE. On the other hand, the latter technique is more efficient for extracting phenols, free fatty acids, lactones and heavier aldehydes, ketones, alcohols and esters. These two extraction methods are therefore complementary and it is desirable to combine both techniques in order to detect the largest number of volatile compounds from cheeses.

Using the DHS, the "dry method" made possible the extraction of a greater number of compounds and in a larger quantity than the "suspension technique" (Canac-Arteaga, Viallon, & Berdagué, 1999, 2000). This effect could be related to the higher solubility of polar compounds in the aqueous phase. A possible risk of the "dry method" is the higher probability of overlapping of two chromatographic peaks, especially if they are very different in size. Nevertheless, a few compounds such as ethyl hexanoate, nonanal and decanal are better extracted using the "suspension technique".

With respect to different cheese varieties, a greater number of esters, free fatty acids, alcohols and phenols were found in the 8-month-ripened Fiore Sardo cheese

than in the 5-month-old Roncal cheese. The latter was characterised by the presence of more 2-propenal, nonanal, decanal, 3-buten-2-one and unsatured hydrocarbons.

Pecorino Sardo cheese presents a different "finger print" of volatiles at different ripening stages (1 and 7 months). It is therefore possible to detect the influence of the ripening process. Some compounds present in the 1-month-old cheese disappeared, e.g. decanal, benzene, octane, propanoic acid ethyl ester, butanoic acid 3-methyl butyl ester and acetoin; other compounds decreased during ripening such as many alcohols, some ketones, 2,3-butanedione, butan-2-one, acetic, butanoic and hexanoic ethyl esters. Many other compounds such as aldehydes, some alkanes, ketones and alcohols appeared or increased as degradation products during ripening.

The diet of the sheep may influence the composition of the volatile fraction. The terpenes (limonene and α-pinene) present in cheese from sheep which grazed on mountain fodders may be due to the consumption of green grass. This occurred in Roncal cheese from the Pyrénées' valley. α-Pinene was also detected in the Fiore Sardo cheese which is produced in a mountainous region of Sardinia, while these compounds do not appear in the Pecorino Sardo cheese manufactured in the hilly area of the island. These explanations are hypotheses; both components also have a possible microbial origin.

Due to the small number of samples considered for this preliminary study (one sample per cheese type and per ripening age), no data are available concerning the natural variability of these cheese varieties.

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