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Solid-phase microextraction of volatile compounds in ''Terrincho'' ewe cheese
Comparison of different fibers

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Abstract

Solid-phase microextraction coupled to gas chromatography–mass spectrometry (GC–MS) was applied to study the volatile compounds in ''Terrincho'' ewe cheese. Six types of fibers were tested and the main extraction parameters were studied. Carboxen–polydimethylsiloxane fiber 75 μm (CAR–PDMS) achieved the most complete profile of ewe cheese volatile compounds. The optimised conditions used for characterization of ''Terrincho'' ewe cheese were: sample vial equilibration at 20 °C for 20 min, followed by CAR–PDMS fiber exposure to the headspace above the sample for 30 min and finally thermal desorption of the adsorbed substances, in the injector port for GC–MS analysis. This technique was a useful tool for the differentiation of 11 ''Terrincho'' ewe cheeses, all taken from the same cheesemaking season with 30 days of ripening but from three different farmhouses, according to their volatile fraction. Results obtained were statistically treated by categorical principal component analysis. Subsequently, 49.15% of the variation in data was due to the first dimension ($k=12.7$) and the second dimension ($k=8.88$) accounted for 34.2% of the total information. Volatile profiles among samples indicated cheese group separation according to farmhouse of production.

Keywords: Cheese; Food analysis; Solid-phase microextraction; Volatile organic compounds

1. Introduction

Proteolysis and lipolysis are among the most important events occurring during cheese ripening, leading to the production of volatile compounds, a final result of particular importance for the consumer. Notable efforts have been made to characterize the volatile profiles of different cheese varieties [1]. ''Terrincho'' ewe cheese is a typical product of the North-Eastern region of Portugal, it is a semi-hard cheese manufactured from raw ewe's milk of the race ''Churra da Terra Quente'' according to the specifications of its Denomination of Origin Regulatory Board D.N. No. 293/93 [2]. Thus, the area and

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conditions for ''Terrincho'' ewe cheese production are well established. It undergoes a minimum of 30 days of ripening and its consumption has increased over the past few years. However, studies related to its volatile fraction are quite scarce, yet are necessary for the maintenance or improvement of quality of this cheese.

Generally, volatile components of cheese are present in trace amounts and require isolation and concentration for subsequent gas chromatography (GC) analysis. Different analytical techniques have been used for isolating and studying the volatile components of cheeses [3–6]. The technique of solid-phase microextraction (SPME) [7] has been successfully used to analyse the composition of volatile compounds of different foods and drinks [8–12], yet there have been few reports of application of SPME in the study and characterization of volatile compounds in cheese [3,5,6]. This technique fits the conditions required for quality control analysis in the dairy industry because it is solvent free, cheap, easy to use, and relatively fast to execute. However, the selection of the best SPME fiber to analyse ewe cheese volatiles requires further research; the choice of fiber depends on the type of volatile compounds present in the food.

The traditional and widely used polydimethyl siloxane fiber coating has very good stability and is usually the first fiber tested; this fiber has very high sensitivity to nonpolar compounds, but not to polar compounds. Since the target volatile cheese components belong to various classes of compounds of different polarity, for example, acids, hydrocarbons, carbonyl compounds, alcohols, terpenoids, and sulfur compounds, other fibers need to be considered. A number of new fibers with a potentially greater range of selectivities prompt the evaluation of their performance in the analysis of ''Terrincho'' ewe cheese components. Such fibers include polydimethylsiloxane (PDMS), polydimethylsiloxane–divinylbenzene (PDMS–DVB), polyacrylate (PA), Carboxen–polydimethylsiloxane (CAR–PDMS), Carbowax–divinylbenzene (CW–DVB), and StableFlex divinylbenzene–carboxen–polydimethylsiloxane (DVB–CAR–PDMS).

Hence, the aim of the present study was 2-fold; to choose the appropriate fiber in order to achieve the most complete profile of ewe cheese volatile compounds (or the most appropriate fiber to extract substances of a certain group with high efficiency) and to optimise SPME conditions in order to have a useful tool for differentiation of ''Terrincho'' ewe cheeses from different farmhouses.

2. Experimental section

2.1. Materials

All chemicals were purchased from Sigma (St. Louis, MO, USA) and were of the highest purity available.

''Terrincho'' ewe cheeses used in the trials for optimisation of the method were purchased from local markets and were stored at 4 °C, until analysis.

Samples from four cheese loaves manufactured in the same cheese vat were analysed. They were collected from three different farmhouses, except for a farmhouse that supplied only three cheese loaves (thus $n=11$ samples). These cheeses were all produced during the same cheese-making season (January) and ripened for 30 days. All cheese samples were coded with a letter (V representing ''Veiguinha'' farmhouse, M for ''Mendonça'' farmhouse, and R for ''Ribeirinha'' farmhouse) and a number (1–4) representing the four cheeses within the same batch.

2.2. Sample preparation

The cheese samples were finely grated; prior to grating, a layer of 0.3 cm was removed from the cheese surface in order to minimize the sampling of those volatile compounds that might have eventually migrated from the environment. For each cheese, a 3 g sample taken was and placed in a 15 ml vial subsequently sealed with PTFE–silicone septa (Supelco, Bellefonte, PA, USA).

2.3. Headspace and SPME

The fibers tested for the extraction of the volatile components were as follows: 100 μm PDMS, 65 μm PDMS–DVB, 85 μm PA, 75 μm CAR–PDMS, 65 μm CW–DVB, and 50 μm StableFlex DVB–CAR–PDMS (Supelco).

The performance of the six fibers was compared by using a ''Terrincho'' ewe cheese, ripened for more than 2 months. The sample vials were equilibrated for 30 min at 20 °C (thermostatically controlled analysis room). Subsequently, the stainless steel needle in which the fiber is housed was pushed through the vial septum, allowing the fiber to be exposed to the headspace of the sample for 20 min., The fiber was then pulled into the needle sheath and the SPME device was removed from the vial and inserted into the injection port of the GC system for thermal desorption. During the injection process the fiber was maintained for 10 min, in the splitless mode.

The fiber that presented the most complete profile of ewe cheese volatile compounds was then used to optimize the SPME conditions. For such purpose, different equilibrium times were tested, viz. 2, 5, 10, 20, 30 and 60 min before the fiber was introduced into the vial: the time of exposure of the fibre was maintained at 20 min. Furthermore, the adsorption kinetics was also evaluated for different exposure times (2, 5, 10, 20, 30 and 60 min) of the fiber in the headspace.

Finally, the study of ''Terrincho'' ewe cheese volatile fraction was carried out using a CAR–PDMS fiber, an equilibrium time of 20 min and an exposure time of 30 min.

2.4. Chromatography

The analyses were performed using a Hewlett-Packard (HP) model 6890 GC system, fitted with a splitless injector suitable for SPME analysis, and an Agilent 5973 mass spectrometry (MS) system. Helium was used as the carrier gas with a flow-rate of 1 ml/min. The components were separated on a SPB-5 capillary column 60 m×0.32 mm, 1.0 μm film thickness (Supelco). The oven temperature program was 5 min at 40 °C and then 3 °C min^{-1} to 200 °C for 5 min.

The injector temperature was set according to the nature of the fiber used: 250 °C for the PDMS, PDMS–DVB, CW–DVB, DVB–CAR–PDMS and PA fibers and 290 °C for the CAR–PDMS fiber. These temperatures, close to the maximum temperature recommended by the manufacturer, allowed us to avoid a significant carry over effect.

Detection was by mass spectrometry on the total ion current obtained by electron impact at 70 eV. The constituents were identified by comparing the experimental spectra with those of the NIST' 98 data bank (NIST/EPA/NISH Massa Spectral Library, version 1.6, USA). Based on the peak resolution, their areas were either calculated from the total ion current or estimated from the integrations performed on selected ions. The resulting peak areas were expressed in arbitrary units of area.

2.5. Statistical analysis

Statistical analyses of results was carried out by categorical principal component analyses using SPSS for windows version 11.5 (SPSS, Chicago, IL), which were conducted to determine the volatile compounds that differentiate ''Terrincho'' cheeses from three different farmhouses. This procedure simultaneously quantified categorical variables while reducing the dimensionality of the data. The optimal-scaling approach allowed to be scaled at different levels.

3. Results and discussion

3.1. Choice of SPME fiber

The PDMS fiber presented the lowest overall sensitivity. The more polar PA fiber can be used for the extraction of volatile free fatty acids, ketones and lactones. However, mixed coating fibers, containing DVB, PDMS, CAR (a porous activated carbon support) or CW (polyethylene glycol), increase retention capacity due to the mutually potentiating effect of adsorption to and distribution within the stationary phase as indicated by the tabulated results. Comparison of the performance of the six fibers tested clearly showed that, in general, mixed coating fibers enabled detection of a wider range of compounds and produced higher signal intensities than did PDMS and PA fibers.

The most complete profile of ewe cheese volatile compounds corresponds to analyses carried out with the CAR–PDMS fiber, which extracted more than 60 compounds at ambient temperature (alkanes, esters,

ketones, carboxylic acids, etc.) with widely ranging polarities and molecular masses.

The CAR–PDMS 75-μm phase is a porous material (micro-, meso-, and macropores from 6 to 50 Å) resulting from a mixture of carbon and PDMS. Given its good performance in the extraction of the highest possible number of ewe cheese volatile compounds, this fiber was selected to characterize the volatile fraction of ''Terrincho'' ewe cheese. These findings were in agreement with other research works performed in dairy products. The CAR–PDMS phase has already been applied in the characterization of cheese of the ''Camembert'' type [5], and in the characterization of off-odours in milk [13].

3.1.1. Acid compounds

It is important to underline the contribution of free fatty acids in cheese aroma, either directly by their aromatic notes, or as precursors of methyl ketones, alcohols, lactones, alkanes and esters [1]. Thus, free fatty acids can be involved in cheese aroma or in a rancidity defect when they are present in very large amounts. The whole range of volatile fatty acids (up to 12 carbon atoms) is usually detected in ewe cheeses. Each compound has a characteristic odorous note [14]. Some octanoic acids associated with the typical flavours of ewe and goat cheeses are not detected in cheeses made with cow's milk [1].

As shown in Table 1, PDMS fiber was not adequate for the extraction of free fatty acids. PA was the fiber that enabled identification of most free fatty acids. This fiber is appropriate for selective adsorption of volatile free fatty acids as reported by Pinho et al. in a previous work [15]. Fibers such as CAR–PDMS, CW–DVB, PDMS–DVB and DVB–CAR–PDMS gave higher overall sensitivity for those fatty acids.

3.1.2. Neutral compounds–alcohols

Different alcohols (generally, primary and secondary) may be found in ''Terrincho'' ewe cheese, these compounds give an alcoholic and/or floral note.

For the extraction of alcohols a CAR–PDMS fiber was the most appropriate (see Table 2), but these compounds were also extracted with the other mixed coating fibers. The PDMS and PA fibers did not display affinity for the extraction of alcohols present in the headspace of ''Terrincho'' cheeses.

Table 1
Acid compounds identified by SPME–GC–MS analysis of the volatile fraction of a sample of ''Terrincho'' ewe cheese according to the type of SPME fiber

Compound	Flavour note[a]	t_R (min)[b]	*Tic*/Ion[c]	Relative areas achieved with different SPME fibers					
				CAR–PDMS	CW–DVB	PDMS–DVB	DVB–CAR–PDMS	PA	PDMS
Ethanoic acid	Vinegar, pungent	10.78	60	100	5	1	6	1	0
Propanoic acid	Vinegar, pungent	15.13	74	100	61	6	nd	4	nd
2-Methyl-propanoic acid	Sweet, applelike, rancid butter	18.46	Tic	22	67	100	87	11	2
Butanoic acid	Rancid, cheesy, putrid, sweaty	21.50	60	96	73	85	100	18	4
Isopentanoic acid		24.07	Tic	55	76	100	88	1	6
2-Methyl-butanoic acid	Fruity, sour, sweaty	24.50	Tic	71	70	100	35	3	nd
Pentanoic acid	Cheesylike, sweaty, rancid, waxy	26.13	Tic	65	51	73	100	10	nd
Hexanoic acid	Pungent, blue, cheese, sour	32.28	Tic	59	82	80	100	57	17
Heptanoic acid		37.45	Tic	nd	nd	nd	nd	100	nd
2-Ethyl-hexanoic acid		39.93	Tic	nd	nd	nd	nd	100	nd
Octanoic acid	Goaty, waxy, soapy, musty, rancid, fruity	42.67	Tic	31	50	27	nd	100	23
Sum of relative areas				598	534	572	515	404	52
No. of identified peaks				9	9	9	7	11	6

[a] The sensory properties of the most characteristic compounds are indicated.
[b] Retention time.
[c] When the resolution was not perfect chromatographic peak areas were not calculated from the total ion current (*Tic*) but were estimated from integrations performed on the specified indicated ions.

Table 2
Alcohol compounds identified by SPME–GC–MS analysis of the volatile fraction of a sample of ''Terrincho'' ewe cheese according to the type of SPME fiber

Compound	Flavour note[a]	t_R (min)[b]	*Tic*/Ion[c]	Relative areas achieved with different SPME fibers					
				CAR–PDMS	CW–DVB	PDMS–DVB	DVB–CAR–PDMS	PA	PDMS
Ethanol	Alcohol, mild	6.33	Tic	100	15	16	20	10	4
Prop-2-en-1-ol		8.45	Tic	42	50	31	100	nd	nd
Propan-1-ol	Alcohol, sweet	8.80	Tic	100	13	49	46	8	6
2-Methylpropan-1-ol	Alcohol, penetrating	11.61	74	100	11	148	59	nd	9
Butan-1-ol	Sweet, fruity	13.43	Tic	100	55	17	28	nd	nd
2-Methoxypropan-1-ol		14.14	Tic	16	9	8	100	nd	nd
Pentan-2-ol	Mild green, fusel oil	15.35	Tic	100	nd	77	46	nd	nd
2-Butanol-3-one		15.99	Tic	82	34	100	4	3	6
3-Methylbutan-1-ol	Fruity, alcohol	17.49	Tic	35	12	100	8	4	8
2-Methylbutan-1-ol		17.73	Tic	39	26	100	27	nd	nd
Butan-1,3-diol		20.18	45	100	11	nd	61	nd	nd
Butan-2,3-diol		20.80	45	3	100	47	nd	nd	nd
Hexan-1-ol	green	25.90	56	22	100	46	50	nd	nd
Heptan-2-ol	easthy oily	27.85	Tic	72	19	100	75	nd	nd
Sum of relative areas				911	454	839	624	25	32
No. of identified peaks				14	13	13	13	4	5

[a] The sensory properties of the most characteristic compounds are indicated.
[b] Retention time.
[c] When the resolution was not perfect chromatographic peak areas were not calculated from the total ion current (Tic) but were estimated from integrations performed on the specified indicated ions.

3.1.3. Ketones and aldehydes

Methyl ketones are the most abundant neutral compounds in the volatile fraction of ''Terrincho'' ewe cheese (see Table 3). Due to their typical odours, methyl ketenes have a key role in the flavour of ripened cheese.

Again, CAR–PDMS was the fiber that extracted more compounds and enabled the highest sensitivity for extraction, followed by the other mixed coating fibers. In general, a lower affinity and sensitivity for extraction of ketones, present in the headspace of cheeses, was obtained using the PDMS and PA fibers (see Table 3).

The most appropriate fiber to extract aldehydes present in ''Terrincho'' ewe cheese headspace was CAR–PDMS (see Table 3).

3.1.4. Esters

Most esters have floral and fruity notes and may contribute to cheese aroma by minimizing the sharpness and bitterness imparted by fatty acids and amines, respectively.

CAR–PDMS and PDMS–DVB were the most sensitive fibers to extract esters from ''Terrincho'' ewe cheese headspace (see Table 4).

3.1.5. Other volatile compounds

Methanediol is referred to as the primary compound contributing to the strong and putrid aroma (such as dimethyl sulfide and dimethyl disulfide), normally associated with cheese. Other sulfur compounds found in cheese are described as having a strong garlic and very ripe cheese odour.

Sulphur compounds and terpene compounds (e.g. limonene and junipene) were efficiently extracted from ''Terrincho'' ewe cheese headspace using CAR–PDMS fiber as shown in Table 5.

Hydrocarbons, such as 1,3-pentadiene and 2,6-dimethyloctene, and benzenic and chloride compounds, such as xilene, toluene, benzene, methylene chloride, trichlorometane and trichloroethylene, were similarly extracted using the CAR–PDMS and DVB–CAR–PDMS fibers (see Table 5).

With respect to the presence of lactones only 4-hydroxy butanoic acid lactone was detected in ''Terrincho'' ewe cheese when using the PA fiber

Table 3
Ketone and aldehyde compounds identified by SPME–GC–MS analysis of the volatile fraction of a sample of ''Terrincho'' ewe cheese according to the type of SPME fiber

Compound	Flavour note[a]	t_R (min)[b]	*Tic*/Ion[c]	Relative areas achieved with different SPME fibers					
				CAR–PDMS	CW–DVB	PDMS–DVB	DVB–CAR–PDMS	PA	PDMS
Ketones									
Propanone	Ethereal, powerful, fruity	6.98	Tic	nd	100	nd	nd	nd	nd
Propan-2-one	Acetone	7.03	Tic	100	68	28	48	5	6
Butan-2,3-dione		9.90	86	100	nd	nd	nd	6	nd
Butan-2-one	Acetone, ethereal	10.26	72	100	2	15	21	1	2
Pentan-2-one	Fruity, acetone, sweet, etheral	14.74	Tic	100	8	54	51	3	4
3-Methylpentan-2-one,		18.73	Tic	24	63	100	67	nd	nd
Hexan-2-one	Floral, fruity	20.94	58	7	100	42	6	nd	nd
Heptan-2-one	Blue cheese, spicy, Roquefort cheese, musty	27.32	Tic	100	17	65	52	1	2
Nonan-2-one	Fruity, musty, floral	39.13	Tic	100	75	44	55	9	3
Sum of abundance %				631	433	348	300	25	18
No. of identified peaks				8	8	7	7	6	5
Aldehydes									
Ethanal	Ethereal, pungent green	5.58	42	nd	100	nd	nd	nd	nd
3-Methyl butanal	Green, malty	13.02	Tic	54	21	100	79	nd	nd
2-Methyl butanal	Green, malty	13.53	86	100	nd	nd	nd	nd	nd
Sum of relative areas				154	21	100	79	0	0
No. of identified peaks				2	2	2	2	2	2

[a] The sensory properties of the most characteristic compounds are indicated.
[b] Retention time.
[c] When the resolution was not perfect chromatographic peak areas were not calculated from the total ion current (TIC) but were estimated from integrations performed on the specified indicated ions.

Table 4
Ester compounds identified by SPME–GC–MS analysis of the volatile fraction of a sample of ''Terrincho'' ewe cheese according to the type of SPME fiber

Compound	Flavour note[a]	t_R (min)[b]	*Tic*/Ion[c]	Relative areas achieved with different SPME fibers					
				CAR–PDMS	CW–DVB	PDMS–DVB	DVB–CAR–PDMS	PA	PDMS
Ethyl ethanoate	Solvent, pineapple, fruity	11.04	Tic	100	36	67	52	nd	nd
Ethyl butanoate	Pineapple, sweet, banana fragrant	21.64	Tic	100	18	8	88	nd	7
3-Methylbutyl ethanoate	Fruity, banana	26.40	Tic	90	nd	100	nd	nd	5
Ethyl-3-methylbutanoate	Fresh cheese	24.97	Tic	100	nd	nd	nd	nd	3
Propyl butirate	Sharp, pungent, rancid, sweaty, sickening	27.72	Tic	56	15	100	94	nd	3
Ethyl hexanoate	Pineapple, banana, apple powerful	33.76	Tic	100	66	100	nd	7	17
Ethyl octanoate	Apricot, win, floral	44.53	Tic	8	10	2	100	nd	5
Sum of relative areas				553	145	377	334	7	41
No. of identified peaks				7	5	6	4	1	6

[a] The sensory properties of the most characteristic compounds are indicated.
[b] Retention time.
[c] When the resolution was not perfect chromatographic peak areas were not calculated from the total ion current (TIC) but were estimated from integrations performed on the specified indicated ions.

Table 5
Other volatile compounds in the SPME–GC–MS chromatogram of ''Terrincho'' ewe cheese

Class	Compound	Flavour note[a]	t_R (min)[b]	*Tic*/Ion[c]	Relative areas achieved with different SPME fibers					
					CAR–PDMS	CW–DVB	PDMS–DVB	DVB–CAR–PDMS	PA	PDMS
S	Methanethiol	Cooked cabbage	5.97	47	100	nd	nd	nd	nd	nd
S	Dimethyl sulfide	Boiled cabbage sulfurous	7.798	Tic	100	50	92	99	nd	nd
A	1,3-Pentadiene		7.944	Tic	100	33	nd	33	nd	nd
C	Methylene chloride		8.123	Tic	100	nd	n.d	nd	nd	nd
C	Trichlorometane		11.286	Tic	100	nd	nd	80	nd	nd
B	Benzene		13.58	86	100	nd	nd	73	nd	nd
C	Trichloroethylene		15.658	130	4	28	nd	100	nd	nd
B	Toluene		19.852	91	13	nd	nd	100	nd	nd
L	4-Hydroxybutanoic acid lactone	Faint, sweet, carame	28.69	Tic	nd	nd	nd	nd	100	nd
A	2,6-Dimethyloctene		31.22	57	12	100	nd	nd	nd	4
T	Limonene	Mild, citrus, sweet, orange lemone	36.11	93	100	48	14	43	nd	nd
T	Junipene		56.28	Tic	100	nd	nd	nd	nd	nd
	Sum of relative areas				829	259	106	528	100	4
	No. of identified peaks				11	6	2	7	1	2

Classes of compounds: S, sulphur compounds; A, alkene compounds; B, benzene compounds; C, chloride compounds; L, lactones; and T, terpenes.

[a] The sensory properties of the most characteristic compounds are indicated.

[b] Retention time.

[c] When the resolution was not perfect chromatographic peak areas were not calculated from the total ion current (TIC) but were estimated from integrations performed on the specified indicated ions.

(see Table 5); this observation is not surprising as the lactones are related to the use of pasteurised milk. Cheeses made with heated milk have been shown to contain more lactones, and in the case of ''Terrincho'' ewe cheese, this is made from raw milk.

3.2. Choice of SPME–GC–MS conditions

3.2.1. Sample vial equilibrium

The sample vials were held at 20 °C for different times (2, 5, 10, 20, 30 and 60 min) to establish equilibrium between headspace and sample prior to exposure of the fiber to headspace for a further 20 min. Although, no significant differences were observed in the qualitative profiles as a function of equilibration time, higher equilibrium periods corresponded to higher peak areas of the different volatile compounds. For example, an equilibrium time of 20 min registered a higher level of volatile compounds already present at 2 min (especially the less volatile ones). Thus, this time was preferred and used in further optimisation studies.

3.2.2. Fiber exposure time

The CAR–PDMS fiber was exposed, to headspace in different vials each containing 3 g of cheese, for 2, 5, 10, 20, 30 and 60 min, respectively. Fig. 1 shows the efficiency of the extraction displayed as the sum of peak areas of total volatile compounds after

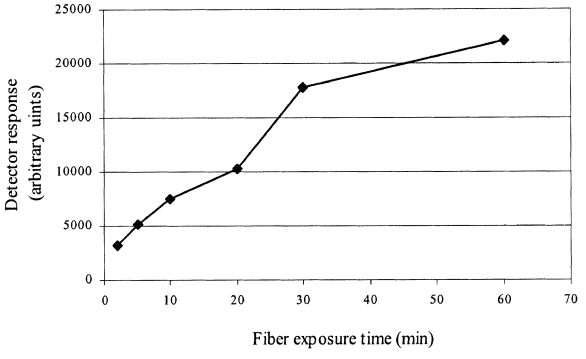

Fig. 1. Effect of adsorption time at room temperature on the extraction efficiency of CAR–PDMS fiber in an ewe cheese sample (y-axis=total volatile compounds expressed as peak area counts $\times 10^6$).

different exposure times of the fiber to the cheese headspace. The rate of extraction was highest during the first 30 min of fiber exposure (see Fig. 2). The rate decreased, thereafter and for some compounds equilibrium seemed to have been established. Nevertheless, for the majority of compounds, their peak areas increased during the whole course of the extraction process, which indicated that equilibrium had not been reached within 60 min. An exposure time of 30 min was chosen.

3.3. Reproducibility of the optimised method

The reproducibility of the method was determined by 10 analyses of the same cheese sample. It was found that the reproducibility depended on the compounds, it ranged between 5 and 15% for acids, between 2.7 and 12.4% for alcohols, between 6.4 and 17.6% for ketones, and between 3.2 and 13.2% for other compounds. Similar values were obtained for solid matrices by other authors [16].

3.4. Application of the SPME–GC–MS method for discrimination of ‘‘Terrincho’’ cheeses from different farmhouses

A categorical principal component analysis (CATPCA) was performed to simplify data from volatile profiles of ‘‘Terrincho’’ cheeses from three different farmhouses. The results have been depicted on a two-dimensional plot (Fig. 2) that explained 83.33% of the total variance. Generally, cheese samples were separated into three groups, according to farmhouse origin (R, M and V). The Dimension 1 (k=12.8) explained 49.15% of the variance in data. The negative segment of the plot for Dimension 1 was related to heptan-2-ol, ethanoic acid, 2-hydroxyethyl propanoate, ethylhexanoate, pentan-2-ol, pentan-2-one, α-pinene and dimethyl-sulfide, while the positive segment of the plot for that Dimension was mainly related to nonan-2-one, 1,2-dimethylbenzene, heptane, 2-methylethyl propanoate, 2-methylpropanoic acid, ethyloctanoate and pentanoic acid. The Dimension 2 was basically related to ethyl ethanoate, 3-methylbutanoic acid, 2-butoxyethanol, 2-methylpropan-1-ol, ethylbenzene, hexanoic acid and octanoic acid, only for the last three, the relationship yielded a negative coefficient.

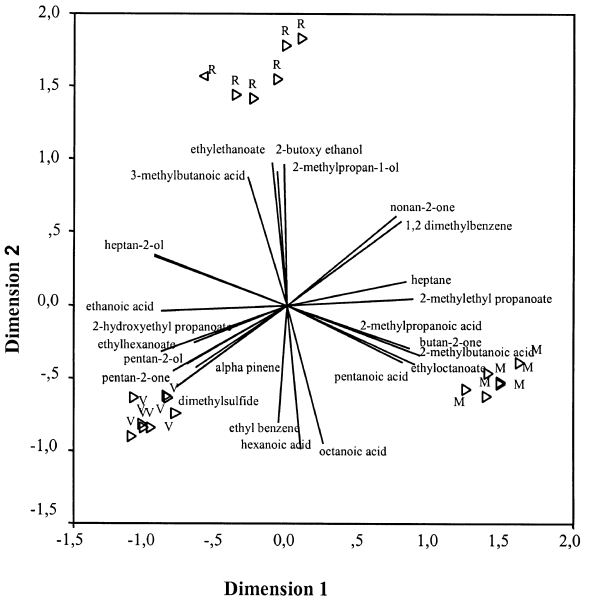

Fig. 2. Categorical principal component biplot showing relationship between cheese samples from three different farmhouses and most import volatile compounds.

4. Conclusions

SPME is a suitable method for the extraction of polar as well as non-polar cheese volatile compounds if the optimal fiber is used. Within the fibers tested the CAR–PDMS fiber was the most appropriate in achieving a complete profile of ewe cheese volatile compounds. However, other fibers can be more appropriate when selective monitoring of substances from a certain group is required. Therefore, extraction conditions should be selected depending on the goals of the study.

Results suggest that the SPME–GC technique could be used for identifying differences in volatile profiles within ‘‘Terrincho’’ ewe cheeses and may provide a means to monitor changes in specific volatiles over the ripening period.

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