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Comparison of dynamic headspace methods used for the analysis of the volatile composition of Spanish PDO ewe's raw milk cheeses

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Abstract – Different dynamic headspace (DHS) methods used for determination of volatile compounds in cheeses were compared. Three different laboratories using gas chromatography coupled to mass spectrometry carried out the analyses of volatile compounds of Spanish Protected Designation of Origin ewe's raw milk cheeses. One laboratory used "purge and trap" (P&T) with thermal desorption of the trap by ultrafast microwave heating (MWH) and the other two laboratories used P&T with thermal desorption of the trap by resistive heating (RH). Each method detected around 78–85 different individual compounds which belonged to different chemical groups, namely, acids, alcohols, ketones, esters, aldehydes, sulphur compounds, hydrocarbons and terpenes. Using the P&T-MWH method, larger percentages of acids and aldehydes in the cheeses were found compared with the P&T-RH methods, whereas the contrary occurred for the percentages of alcohols and ketones. Differences in the percentages of some volatile compounds such as ketones, alcohols and acids between the laboratories using P&T-RH under different analytical conditions were also recorded. The results pointed out differences in the selectivity for the extraction of various volatile compounds from cheese samples according to the DHS methods used, particularly when using tenax or graphitised carbon as trap absorbents. In spite of these differences, both P&T-MWH and P&T-RH methods were equally satisfactory for the comparison of the different cheese varieties. However, each of the DHS methods was more suitable for the characterisation of the volatile compounds of a particular cheese type.

cheese / volatile compound / dynamic headspace analysis / inter-laboratory study

摘要 – 动态顶空法分析西班牙原产地名号保护的羊奶干酪中挥发性化合物的对比研究。本文比较了不同的动态顶空法测定干酪中挥发性化合物。分别在 3 个不同的实验室, 采用气-质联机方法分析了西班牙原产地名号保护 (PDO)、原料奶未经巴氏杀菌的羊奶干酪中挥发性化合物。其中一个实验室采用“吹扫-捕集”(P&T) 方法捕集挥发性气体, 然后用超

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速微波加热 (MWH) 的方法解析气体, 另外两个实验室也采用“吹扫-捕集”方法捕集挥发性气体, 但用热电阻加热 (RH) 方法解析气体。每种方法都能检测出 78–85 种不同的挥发性化合物, 这些化合物分属于酸、醇、酮、酯、醛、含硫化合物、烃类、萜烯化合物。两种方法测得结果的比较分析表明, 采用吹扫-捕集-微波加热方法测定的挥发性化合物中酸和醛占的比例较大, 而采用吹扫-捕集-热电阻加热方法所测定挥发性化合物中醇和酮所占的比例较大。同时比较了均使用吹扫-捕集-热电阻加热方法的两个实验室的分析结果, 由于分析条件的不同, 测定结果中一些挥发性化合物, 如酮、醇和酸占总挥发性化合物的百分比有一定的差异。试验结果表明由于不同的动态顶空法对于酪样品中挥发性化合物提取物的选择性不同, 使得分析结果存在一定的差异, 特别是使用 *tenax* 和石墨作吸附剂时, 这种差异非常显著。尽管两种方法的测定结果之间存在一定的差异, 经对不同种类干酪的测定结果进行比较和分析, 测定结果还是非常满意的。然而每种动态顶空法都有其适合于特定类型干酪中挥发性化合物的分析。

干酪 / 挥发性化合物 / 动态顶空分析 / 实验室研究

Résumé – Comparaison des méthodes d'extraction par espace de tête dynamique employées pour l'analyse des composés volatils des fromages espagnols d'Appellation d'Origine Protégée produits à partir de lait cru de brebis. Différentes méthodes d'extraction par espace de tête dynamique pour l'analyse des composés volatils du fromage ont été comparées. Trois laboratoires utilisant la chromatographie en phase gazeuse couplée à la spectrométrie de masse ont analysé cinq variétés de fromages espagnols d'appellation d'origine protégée fabriqués à partir de lait cru de brebis. Un des laboratoires a employé un système de « purge and trap » (P&T) avec désorption thermique du piège par chauffage ultra rapide par micro-ondes (CMO) et les autres un système de P&T avec désorption thermique du piège par chauffage résistif (CR). Chaque méthode a permis de détecter environ 78–85 composés individuels différents, qui appartenaient à différents groupes chimiques, tels que acides, alcools, cétones, esters, aldéhydes, composés soufrés, hydrocarbures et terpènes. Des pourcentages plus élevés d'acides et d'aldéhydes, et moins élevés d'alcools et de cétones, ont été trouvés en utilisant la méthode P&T-CMO comparativement aux méthodes P&T-CR. De plus, des différences dans les pourcentages de certains composés volatils, tels que cétones, alcools et acides ont également été observées entre les laboratoires utilisant le système de P&T-CR dans des conditions différentes. Les résultats soulignent des différences en termes de sélectivité d'extraction des volatils à partir des échantillons de fromage selon la technique d'extraction employée, et en particulier selon la nature de l'absorbant du piège (*tenax* ou charbon). Malgré ces différences, les deux méthodes P&T-CMO et P&T-CR se sont révélées également satisfaisantes pour comparer différents variétés de fromages. Cependant, chacune des méthodes d'extraction était plus appropriée pour caractériser les composés volatils pour un type de fromage en particulier.

fromage / composé volatil / espace de tête dynamique / étude inter-laboratoire

1. INTRODUCTION

The unique flavour of Protected Designation of Origin (PDO) cheeses is most important for the successful commercialisation of these products. One of the principal aims of the PDO Regulatory Boards is to characterise the typical flavour of their cheeses in order to offer consumers a unique product which can be clearly differentiated from other cheese varieties. During the last few years, it has been suggested that the volatile composition and flavour sensory properties can be used to discriminate between different cheese varieties [4, 12, 13, 29]. Cheeses may contain many volatile compounds such as acids, alcohols, carbonyl

compounds, esters, lactones, furans, nitrogen-containing compounds, sulphur and phenolic compounds, hydrocarbons and terpenes, which differ quantitatively from one variety to another [11]. These compounds are often analysed by gas chromatography (GC) coupled to mass spectrometry (MS), but it is always necessary to include a prior step involving the extraction and pre-concentration of the volatile fraction [52]. Several authors have pointed out that there is no ideal method for the extraction, concentration and injection of the volatile compounds into the chromatographic column, and that the results of the analysis will be different according to the technique used [32]. One critical aspect for cheese researchers

is to select the most suitable extraction technique which allows one to obtain a volatile profile most closely related to the sensory properties of the cheese [28]. Numerous techniques such as simultaneous distillation-extraction [13, 21], high-vacuum distillation [20, 38], supercritical fluid extraction [26], static headspace [14, 41], dynamic headspace (DHS) [49, 51], solid-phase microextraction [30, 31], and direct thermal desorption [50] have been used for the extraction of flavour compounds in cheese [32]. Nowadays DHS is being frequently used [2, 17, 28, 47, 48, 51]. In this technique, the volatile components of the gas phase are continuously removed and concentrated in a trap or absorbed onto an inert support. This headspace system is often referred to as “purge and trap” (P&T) [39]. Thermal desorption of the trap by resistive heating (RH) is extensively used to transfer the volatile components onto the capillary column, cryofocusing being recommended to achieve a narrow injection band [2, 47, 49]. Thermal desorption of the trap by ultrafast microwave heating (MWH) has also been used to very rapidly transfer the volatile components into the capillary column without the need for cryofocusing [1, 3, 5, 45]. P&T-RH has been compared with other techniques, particularly with SDE, for the analysis of volatiles of cheese [8, 27, 50]. However, few studies have focused on the comparison between different DHS methods. Some authors have concluded that cheese volatile analysis carried out by different DHS methods yield different quantitative and even qualitative results [5, 49].

Idiazabal, Manchego, Roncal, Zamorano and La Serena cheeses are the Spanish PDO cheese varieties made from raw ewe’s milk [19]. Only Manchego and Zamorano cheeses may also be manufactured from pasteurised milk. The first four varieties are semi-hard cheeses coagulated with animal rennet, whereas La Serena cheese is a soft cheese variety coagulated with vegetable rennet [33–37]. The main characteristics of these PDO cheeses are shown in Table I. Studies on the volatile composition of Idiazabal, Manchego, Roncal, Zamorano and La Serena cheeses are scarce. Manchego

cheese is the best known among the five varieties, but few studies have described the volatile composition of cheeses made from raw ewe’s milk [15, 21, 51]. Very few papers have described the volatile composition of Idiazabal [26], Zamorano [16], Roncal [25, 42] and La Serena cheeses [6, 7].

The objective of the present study was to compare the results obtained by three different laboratories using different DHS methods in the GC-MS analysis of the volatile compounds of these PDO cheeses. One laboratory used semi-automatic P&T-MWH, and the other two laboratories used automatic P&T-RH.

2. MATERIALS AND METHODS

2.1. Cheese samples

Two batches of each PDO cheese were produced during two consecutive weeks in April. Cheeses were manufactured according to the rules approved by the PDO Regulatory Councils for the production of Idiazabal, Manchego, Roncal, Zamorano and La Serena cheeses [33–37]. All cheeses were sampled for analyses after six months of ripening except for La Serena cheeses which were sampled after two months of ripening (Tab. I). Two cheeses per batch and per variety were taken as duplicate samples. Cheeses were cut into three sectors and one sector of each cheese was transported in portable coolers (3–4 °C) to each of the three laboratories collaborating in this study. Cheese sectors were wrapped in aluminium foil, vacuum-packed and stored at –40 °C until analysis. Prior to volatile analysis vacuum-packed cheese sectors were thawed overnight at 5 °C. Samples from the centre of the cheese sectors were taken for volatile analyses.

2.2. Analysis of volatile compounds

Three laboratories which were using DHS directly coupled to GC-MS for the analysis of the volatiles of the cheeses collaborated in this study. One of the laboratories analysed the volatile compounds by semi-automatic P&T-MWH (method *A*), and the other two laboratories used automatic P&T-RH (methods *B* and *C*).

Table 1. Main characteristics of the Spanish PDO ewe's raw milk cheeses studied.

Variety	PDO location	Type of cheese	Sheep breed	Rennet	Starter culture	Coagulation		Cutting	Ripening		
						T (°C)	t (min)		T (°C)	RH (%)	t (days)
Idiazabal ^a	Basque Country region (Northern Spain)	semi-hard	Latxa	natural lamb rennet paste	homo-fermentative mesophilic LAB	28–32	20–45	milling to 5–10 mm grain, stirring and scalding at 36–38 °C for 25 min	8–10	85–90	180
Roncal ^b	Roncal valley (Northern Spain)	semi-hard	Latxa	bovine rennet	homo-fermentative mesophilic LAB	30–37	30–60	crude milling, stirring and scalding at 38 °C for 30 min	10–12	85–90	180
Manchego ^c	La Mancha region (Central Spain)	semi-hard	Manchega	bovine rennet	homo-fermentative mesophilic LAB	28–32	45–60	milling to 5–10 mm grain, scalding at 36–38 °C for 30–40 min	10–12	80–90	180
Zamorano ^d	Zamora province (Western Spain)	semi-hard	Castellana	lamb liquid rennet	homo-fermentative mesophilic LAB	28–32	30–45	milling to 5–10 mm grain and scalding at 38 °C for 35–40 min	10–12	80–90	180
La Serena ^e	Extremadura Region (South-western Spain)	soft	Merino	vegetable rennet from <i>Cynara cardunculus</i>	no starter	26–30	50–75	milling to 10–20 mm grain, stirring for 30 min without scalding	5–8	85–90	60

T: temperature; t: time; RH: relative humidity; LAB: lactic acid bacteria.

^a Refs: [34, 43]; ^b Refs: [24, 33]; ^c Refs: [15, 37]; ^d Refs: [18, 35]; ^e Refs: [36, 46].

2.2.1. Method A

One hundred grams of cheese were homogenised in an analytical blender at 10–12 °C. An aliquot (15 g) of the grated cheese was placed in a 50-mL gas washing flask. Volatile compounds were extracted with a CL-2 purge system (Chromlab, Barcelona, Spain) using ultrapure helium as purge gas and were trapped onto a graphitised carbon cartridge (1-010R, Reaktorik, Meyrin, Switzerland). The DHS conditions were: 100 mL·min⁻¹ purge gas flow for 30 min, at 40 °C. The volatiles concentrated in the carbon cartridge were desorbed with MW-1A microwave desorption equipment (Rektorik) at 550–590 × 10⁻⁶ A·h desorption energy for 10 s. The MW-1A desorption equipment was connected to an 8000 series GC apparatus coupled to an 800 MD MS detector (Fisons Instruments, Milan, Italy). The transfer line from the desorption equipment to the GC oven was held at 250 °C. Analyses were performed using a SUPELCOWAXTM (Supelco, Bellefonte, Palo Alto, USA) capillary column (60 m long; 0.25 mm ID) coated with cross-linked polyethylene glycol (0.25 µm film thickness). Oven temperature was initially held at 40 °C for 10 min, then increased to 240 °C at a rate of 5 °C·min⁻¹, and finally held at 240 °C for 15 min. The carrier gas (helium) flow rate was 1.0 mL·min⁻¹ and the split ratio 1:5. The transfer line from the GC apparatus to the MS detector was held at 250 °C. The MS detector operated in full scan mode (total ion chromatogram at 1.85 scans·s⁻¹) from 19 to 250 amu. Ionisation was performed by electron impact at 70 eV and the source temperature was 200 °C. Peak identification was done by comparing the mass spectra with the NBS (National Bureau of Standards, USA) and NIST (National Institute of Standards and Technology, USA) libraries, and by comparison of their retention factors with authentic standards when available. The retention factor (*k*) for one peak was calculated applying the formula (1):

$$k = (t_R - t_M) / t_M \quad (1)$$

where *t_R* and *t_M* are the retention times for the peak and for an unretained peak, respectively. Quantification was carried out by the

total ion current. Arbitrary units (peak area) were used.

2.2.2. Method B

Ten grams of cheese were homogenised in an analytical blender at 10–12 °C with 10 g of Na₂SO₄ and 100 µL internal standard aqueous solution prepared with 13 mmol·L⁻¹ borneol (Sigma-Aldrich Química), and the mixture was placed in a suitable U vial (needle sparger). Volatile compounds were extracted using ultrapure helium as purge gas in a 4460A automatic P&T apparatus (O.I. Analytical, College Station, USA). The DHS conditions were: 40 mL·min⁻¹ purge gas flow for 20 min at 40 °C. Volatile compounds were concentrated in a Tenax trap (O.I. Analytical) at room temperature. The transfer line and valves were kept at 120 and 180 °C, respectively. The volatiles were desorbed from the trap at 220 °C for 1 min directly into the GC injection port. The analysis of the volatile compounds was carried out in a HP 6890 GC-MS apparatus equipped with a HP INNOWAX (Agilent Technologies, Las Rozas, Spain) capillary column (60 m long; 0.25 mm ID) coated with cross-linked polyethylene glycol (0.25 µm film thickness). Oven temperature was initially held at 32 °C for 7 min, then increased to 220 °C at a rate of 6 °C·min⁻¹, and finally held at 220 °C for 5 min. The carrier gas (helium) flow rate was 1.5 mL·min⁻¹ for 1 min and then at a constant flow rate of 1 mL·min⁻¹, with a split ratio of 1:30. Injection port temperature was 200 °C. The transfer line from the GC apparatus to the MS detector was held at 220 °C. Detection was performed with the HP 5973 MS detector operating in full scan mode (total ion chromatogram at 3 scan·s⁻¹) from 19 to 250 amu. Ionisation was performed by electron impact at 70 eV and the detector and source temperatures were 108 and 220 °C, respectively. Peak identification was done by comparing the mass spectra with the Wiley 275 library (Wiley & Sons Inc., Germany), and by comparison of their retention factors (*k*) with authentic standards when available. Quantification was carried out by the total ion current, with reference to the borneol peak. Arbitrary units (peak area) were used.

2.2.3. Method C

Fifteen grams of cheese were homogenised in an analytical blender at 10–12 °C with 20 g Na₂SO₄ and 75 µL internal standard aqueous solution prepared with 0.5 mg·mL⁻¹ cyclohexanone (Sigma-Aldrich Química, Alcobendas, Spain). An aliquot (2.25 g) of the mixture was subjected to DHS using ultrapure helium as purge gas in a HP 7695 automatic P&T apparatus (Hewlett-Packard, Palo Alto, USA). The DHS conditions were: 45 mL·min⁻¹ purge gas flow for 20 min at 50 °C, with 10 min of previous equilibration. Volatile compounds were concentrated in a Tenax trap (Tekmar, Cincinnati, USA) maintained at 25 °C and 6.5 psig back pressure. The transfer line, moisture trap and valves were kept at 200 °C. The trap was subjected to dry purge for 0.5 min and the volatile compounds were desorbed for 1 min at 230 °C directly into the GC injection port. The analysis of the volatile compounds was carried out in a HP 6890 GC-MS apparatus equipped with a HP INNOWAX capillary column as in method **B**. Oven temperature was initially held at 45 °C for 17 min, then increased to 110 °C at a rate of 4 °C·min⁻¹, then held at 110 °C for 10 min, and finally increased to 240 °C at a rate of 15 °C·min⁻¹. The carrier gas (helium) flow rate was 1.4 mL·min⁻¹ and the split ratio 1:20. Injection port temperature was 220 °C. The transfer line from the GC apparatus to the MS detector was held at 240 °C. Detection was performed with the HP 5973 MS detector as in method **B** operating in full scan mode from 33 to 200 amu, and the detector and source temperatures were 150 and 230 °C, respectively. Peak identification and quantification were done as described in method **B** using cyclohexanone as the reference peak.

2.3. Statistical analysis

The SPSS statistical package, version 11.0 (SPSS Inc., Michigan, USA) was used for the statistical analysis. Analysis of variance (ANOVA) and multiple comparison tests were applied to determine the presence of significant ($P \leq 0.05$) differences in the volatile composition of each type of PDO

sample cheese analysed by the different DHS methods. Stepwise discriminant analysis was applied to classify the cheese samples according to the method used. Also, discriminant analyses were applied separately for each method to classify the cheese samples according to the PDO cheese variety. Wilk's lambda criterion was used for selecting discriminant variables [22].

3. RESULTS AND DISCUSSION

Table II lists the volatile compounds detected in the headspace of all the cheese samples analysed by the three laboratories using the different DHS methods (**A**, **B** and **C**). A total of 137 volatile compounds were detected by all three methods combined. These compounds belonged to different chemical groups, namely: methyl, ethyl, propyl, butyl, higher alkyl and branched alkyl esters; alkanes, aromatic and unsaturated hydrocarbons; primary, secondary, branched-chain, oxy ether and aromatic alcohols; straight, branched-chain and aromatic aldehydes; methyl, propyl and aromatic ketones; diketones; straight and branched-chain acids; sulphur compounds; and terpenes. Esters, hydrocarbons and alcohols were chemical families with a large number of different individual compounds (40, 30 and 23 compounds, respectively), whereas less than 15 compounds were found for chemical families such as aldehydes (14), ketones (14), acids (10), sulphur compounds (3) and terpenes (3) (Tab. II). Each method detected around 78–85 different individual compounds which varied with the analytical conditions used. Thus, there were 65 different compounds between methods **A** and **B**, 72 different compounds between methods **A** and **C**, and 55 distinct compounds between methods **B** and **C**. Therefore, the volatile compositions reported by the three methods were very different (Tab. II). Only 39 of the 137 compounds were detected by each of the three methods, 33 of the 137 compounds were detected by two of the three methods, whereas the remaining 65 compounds were detected by one of the methods only (not always the same one). Esters and hydrocarbons were the volatile compounds that varied the most

Table II. Volatile compounds found in the Spanish PDO cheeses made from ewe's raw milk using different DHS methods.

Compounds	Method A ^a		Method B ^b		Method C ^b	
	k	I	k	I	k	I
<i>Methyl esters</i>						
methyl ethanoate		ND	34.10	T	38.48	P
methyl butanoate		ND		ND	85.48	T
methyl 2-hydroxy-4-methyl-pentanoate		ND	127.20	T		ND
methyl hexanoate		ND		ND	150.10	P
<i>Ethyl esters</i>						
ethyl ethanoate	39.38	T	40.95	P	49.90	P
ethenyl ethanoate	54.14	T		ND		ND
ethyl propanoate		ND	51.90	T	72.90	P
ethyl 2-methylpropanoate		ND	54.10	T	76.19	T
ethyl butanoate	63.21	P	60.15	P	105.91	P
ethyl 2-methylbutanoate		ND		ND	111.33	T
ethyl 3-methylbutanoate		ND	66.10	T	116.19	T
ethyl pentanoate		ND		ND	136.62	T
ethyl hexanoate	92.66	P	97.00	P	160.91	P
ethyl heptanoate	104.72	T	109.50	P		ND
ethyl octanoate	116.79	P	121.50	T	217.81	P
ethyl decanoate		ND		ND	239.62	P
ethyl benzoate		ND		ND	244.52	T
<i>Propyl esters</i>						
propyl ethanoate	52.79	T		ND	81.00	P
propyl butanoate	76.17	T		ND	133.19	P
propyl 3-methylbutanoate		ND		ND	141.57	T
propyl hexanoate		ND	94.75	T	183.62	T
<i>Butyl esters</i>						
butyl ethanoate	68.66	T	72.65	T	118.10	P
butyl 2-methylpropanoate		ND		ND	139.48	P
butyl butanoate	90.90	T		ND	152.67	T
butyl hexanoate		ND		ND	213.62	T
butyl octanoate	116.79	T		ND		ND
<i>Higher alkyl esters</i>						
hexyl ethanoate	106.14	T		ND		ND
hexyl hexanoate	122.45	T		ND		ND
heptyl hexanoate		ND	108.50	T		ND
<i>Branched alkyl esters</i>						
1-methylpropyl ethanoate	55.07	T	57.85	T	85.76	P
2-methylpropyl ethanoate		ND		ND	97.10	T

Table II. Continued.

Compounds	Method <i>A</i> ^a		Method <i>B</i> ^b		Method <i>C</i> ^b	
	k	I	k	I	k	I
3-methylbutyl ethanoate	76.66	T	75.15	T	133.19	P
2-methylpropyl 2-methylpropanoate		ND	81.50	T		ND
1-methylpropyl butanoate		ND		ND	134.81	T
2-methylpropyl butanoate	77.14	T		ND	142.43	T
3-methylbutyl butanoate		ND	94.90	T		ND
1-methylpropyl hexanoate		ND		ND	183.86	T
2-methylpropyl hexanoate	103.86	T	108.65	T	193.76	T
2-butoxyethyl ethanoate	119.52	T		ND		ND
3-methylbutyl hexanoate		ND		ND	221.33	T
Total number of esters (40)	18		18		30	
<i>Alkanes</i>						
<i>n</i> -pentane		ND	17.80	P		ND
2-methylpentane		ND	18.45	T		ND
3-methylpentane		ND	18.75	T		ND
<i>n</i> -hexane	22.76	P	19.00	P	21.43	T
2-methylhexane		ND	20.04	T		ND
3-methylhexane		ND	20.85	T		ND
cyclohexane		ND	21.00	T		ND
<i>n</i> -heptane	24.31	P	21.70	P	24.52	T
1,3-dimethylcyclopentane		ND	22.35	T		ND
1,2-dimethylcyclopentane		ND	22.65	T		ND
methylcyclohexane		ND	25.60	T		ND
<i>n</i> -octane	29.34	P	27.95	P	32.57	T
2,2-dimethylbutane	70.38	T		ND		ND
5-methylundecane	112.14	T		ND		ND
<i>n</i> -tetradecane		ND	116.70	T		ND
<i>Aromatic hydrocarbons</i>						
benzene	46.62	P		ND		ND
toluene	63.55	P		ND	107.29	T
styrene		ND	102.10	T		ND
ethyl benzene		ND		ND	134.90	T
<i>p</i> -xylene		ND		ND	136.81	T
<i>m</i> -xylene	84.48	T		ND	138.62	T
<i>o</i> -xylene		ND		ND	150.43	T
indene	124.14	T		ND		ND
naphthalene	149.93	T		ND		ND
<i>Unsaturated hydrocarbons</i>						
2-methyl-1-propene	21.10	T		ND		ND

Table II. Continued.

Compounds	Method A ^a		Method B ^b		Method C ^b	
	k	I	k	I	k	I
1,3-pentadiene		ND	19.40	T		ND
1,4-pentadiene	23.24	T		ND		ND
1-heptene	31.03	T		ND		ND
3-octene	32.79	T	29.65	T	39.38	T
2-octene	34.14	T	32.95	T		ND
Total number of hydrocarbons (30)	15		17		9	
<i>Primary alcohols</i>						
ethanol		ND	44.50	P	64.71	P
1-propanol	64.24	T	63.05	P	106.24	P
2-propen-1-ol		ND	77.10	T	130.29	T
1-butanol	81.14	P	84.85	P	138.57	P
1-pentanol	95.45	T		ND	163.86	P
1-hexanol	108.28	P	112.50	T	193.33	P
1-heptanol	125.86	T		ND		ND
1-octanol		ND	135.10	T		ND
<i>Secondary alcohols</i>						
2-propanol	45.48	P	47.60	T	61.05	P
2-butanol	61.72	P	60.95	P	102.33	P
2-pentanol	77.52	P	77.50	P	131.62	P
2-hexanol	98.34	T	95.35	T	156.81	P
2-heptanol	103.93	P	108.20	T	181.67	P
<i>Branched-chain alcohols</i>						
2-methyl-1-propanol		ND	73.00	P	124.00	P
3-methyl-2-pentanol		ND	90.85	T		ND
3-methyl-1-butanol	90.24	P	93.40	P	150.05	P
3-methyl-3-buten-1-ol	95.69	T	97.90	P	164.33	T
3-methyl-2-buten-1-ol	104.52	T	107.95	T	183.43	T
2-ethyl-1-hexanol	121.52	T	128.00	T		ND
<i>Oxy ether alcohols</i>						
2-ethoxyethanol	99.41	T	95.75	T		ND
2-butoxyethanol	114.34	P	118.90	T	222.00	T
<i>Aromatic alcohols</i>						
phenol		ND	178.90	T	274.00	T
2-phenyl-1-ethanol		ND	170.85	T		ND
Total number of alcohols (23)	16		21		17	
<i>n-Aldehydes</i>						
ethanal	24.93	P	21.15	P	25.48	P

Table II. Continued.

Compounds	Method A ^a		Method B ^b		Method C ^b	
	k	I	k	I	k	I
propanal		ND	25.70	T	33.14	T
2-propenal		ND	30.95	T	41.67	T
<i>n</i> -butanal	37.41	T	38.50	T	47.43	P
<i>n</i> -pentanal	62.28	T		ND		ND
<i>n</i> -hexanal	70.31	T		ND	121.43	P
<i>n</i> -heptanal	86.45	T		ND		ND
<i>n</i> -octanal	108.76	T		ND		ND
<i>n</i> -nonanal	113.17	T	116.75	P	210.00	P
<i>n</i> -decanal	124.24	T		ND		ND
<i>Branched-chain aldehydes</i>						
2-methylpropanal		ND	27.25	T	35.81	T
2-methylbutanal		ND		ND	56.90	T
3-methylbutanal		ND	40.30	P	58.24	P
<i>Aromatic aldehydes</i>						
phenylethanal	ND		ND		241.86	T
Total number of aldehydes (14)	8		7		10	
<i>Methyl ketones</i>						
2-propanone	30.93	T	27.7	P	36.38	P
2-butanone	41.17	P	39.10	P	53.23	P
3-buten-2-one	48.28	T	51.35	T		ND
2-pentanone	53.31	P	51.55	P	81.95	P
3-methyl-2-pentanone		ND	62.45	T		ND
4-methyl-2-pentanone	59.28	T		ND		ND
2-hexanone	70.10	P	73.90	P		ND
2-heptanone	85.90	P	86.75	P	149.29	P
2-octanone	100.10	T		ND		ND
3-hydroxy-2-butanone	101.24	T	104.00	P	176.00	P
2-nonanone	112.31	P	117.40	P	208.52	P
<i>Propyl ketones</i>						
4-nonanone	104.72	T		ND		ND
<i>Aromatic ketones</i>						
1-phenylethanone	140.79	T		ND	242.48	T
<i>Diketones</i>						
2,3-butanedione		ND	52.45	P	83.27	P
Total number of ketones (14)	12		10		8	
<i>n-Acids</i>						
ethanoic acid	118.48	P	125.60	P	221.00	T

Table II. Continued.

Compounds	Method A ^a		Method B ^b		Method C ^b	
	k	I	k	I	k	I
<i>n</i> -propanoic acid		ND	134.10	P		ND
<i>n</i> -butanoic acid	135.76	P	144.15	P	238.95	T
<i>n</i> -pentanoic acid	145.68	P		ND		ND
<i>n</i> -hexanoic acid	155.41	P	164.65	T	251.48	T
<i>n</i> -octanoic acid		ND	180.95	T		ND
<i>Branched-chain acids</i>						
2-methylpropanoic acid	129.97	P	137.95	P	233.81	T
3-methylbutanoic acid	139.66	P	148.20	P	241.62	T
2-methylbutanoic acid	146.24	T		ND		ND
2-ethylhexanoic acid		ND	173.55	T		ND
Total number of acids (10)	7		8		5	
<i>Sulphur compounds</i>						
carbon disulphide		ND	21.90	P	27.52	T
dimethyl sulphide		ND		ND	28.91	T
dimethyl disulphide		ND	66.90	T	119.14	T
Total number of sulphur compounds (3)	0		2		3	
<i>Terpenes</i>						
α -pinene		ND	63.00	T	98.29	P
<i>D</i> -limonene	86.93	T	91.50	P	153.10	P
<i>p</i> -cymene	106.79	T		ND	171.71	T
Total number of terpenes (3)	2		2		3	
Total number of volatile compounds (137)	78		85		84	

^a Method using P&T-MWH.

^b Methods using P&T-RH; k: retention factor; I: identification; P: positively identified by comparison with k and mass spectra of authentic standards; T: tentatively identified on the basis of the mass spectra libraries; ND: not detected.

according to the DHS method used. Only 8 of the 40 esters, and 4 of the 30 hydrocarbons were detected by the three methods, whereas 22 esters and 23 hydrocarbons were detected by only one of the methods (not always the same one). A larger number of individual esters was detected with method **C** compared with methods **A** and **B**, probably due to the higher purge temperature used in method **C** (at 50 °C) compared with methods **A** and **B** (both at 40 °C). The largest numbers of individual hydrocarbons

were detected by methods **A** and **B** (15 and 17 hydrocarbons, respectively) (Tab. II).

Alcohols and acids also showed variations according to the DHS method used. Only 12 of the 23 alcohols, and 5 of the 10 acids were detected by each of the three methods, whereas 4 alcohols and 5 acids were detected by only one of the methods (not always the same one). The largest number of individual alcohols was detected by method **B**. It was remarkable that all secondary alcohols were detected by the three

methods, and that no aromatic alcohol was detected by method **A**. The total number of acids detected by each of the three methods was between 5 and 8 (Tab. II).

Only 9 of the 28 carbonyl compounds were detected by the three methods, whereas 6 aldehydes and 4 ketones were detected by only one of the methods (not always the same one). Most individual carbonyl compounds were methyl ketones and *n*-aldehydes. The total number of aldehydes detected by each of the three methods was between 7 and 10, and that for ketones was between 8 and 12 (Tab. II). No sulphur compounds were detected by method **A**, whereas all terpenes were always detected at least by two of the three methods (Tab. II).

Tables III–VII list the mean percentages of the volatile compounds, grouped by chemical families, which were found in the headspace of the five PDO cheeses analysed by each of the three methods. Ethanoic acid, *n*-butanoic acid and 2-butanol were quantified individually because of their large percentage found in some of the cheese varieties. Large differences in the percentages of the volatile compounds of each type of cheese were recorded between the method **A** which used P&T-MHW and the other two methods **B** and **C** which used P&T-RH. Large percentages of acids in Idiazabal (90.3%), Roncal (80.6%), and Zamorano (77.9%) cheeses were found with method **A**, whereas large percentages of alcohols (72–92%) were found with methods **B** and **C** in the same cheeses (Tabs. III–V). As has been previously published, considerable amounts of short-chain free fatty acids have been reported in ripened Idiazabal and Roncal cheeses. Several authors have reported that the content of short-chain fatty acids in Idiazabal cheese after six months of ripening was around 20–30% of the total concentration of free fatty acids [9, 10], and that short-chain fatty acids were the main volatile compounds analysed by simultaneous distillation extraction (SDE) in Roncal cheese after five months of ripening [27]. The percentage of acids was also higher when the volatiles of Manchego and La Serena cheeses were analysed by P&T-MWH (method **A**) compared with P&T-RH (methods **B** and **C**) (Tabs. VI and VII). It has been reported that short-chain

free fatty acids were present in low concentrations in ripened Manchego cheese [44], and this is in agreement with the lower percentage of acids found in this PDO cheese (9.4%) by method **A**. Although no data on free fatty acid composition of Zamorano and La Serena cheeses has been published, it could be indicated that the extraction of volatile acids by semi-automatic P&T-MWH (used in method **A**) was larger than that of automatic P&T-RH (used in methods **B** and **C**). These results could be explained by the different nature of the trap absorbent. The graphitised carbon used by method **A** shows high specific surface area with a large number of active sites for polar compounds. Thus, polar volatile compounds such as short-chain fatty acids are strongly retained in its surface. However, tenax which was used by methods **B** and **C** is a hydrophobic porous polymer with low specific surface area [40]. On the other hand, it must be indicated that tenax is not suitable for desorption by MWH because of its poor heating capability by microwaves [43].

The three methods found large percentages of alcohols in Manchego cheese, although significant differences ($P \leq 0.05$) were recorded among them (61.7, 81.8, and 94.3% of total alcohols, respectively, for methods **A**, **B** and **C**).

A large percentage of aldehydes (58.4%) was found by method **A** in La Serena cheese, whereas large percentages of alcohols (around 85%) were found in this type of cheese with methods **B** and **C**. It was remarkable that aldehydes constituted the main volatile compounds of the aroma profile of La Serena cheese when volatiles were analysed by P&T-MWH (Tab. VII). The percentages of aldehydes found in the other PDO cheeses were also higher when volatile compounds were analysed with method **A** compared with methods **B** and **C** (Tabs. III–VI) because the aldehydes were strongly retained by graphitised carbon.

In general, larger percentages of ketones were detected by methods **B** and **C** than by method **A**, whereas larger percentages of esters were found in the PDO cheese varieties with method **A**, compared with methods **B** and **C**. Except for Manchego cheese, the percentages of hydrocarbons in the PDO cheese varieties found by each of the

Table III. Percentage (means \pm SD; $n = 4$) of the volatile compounds found in Idiazabal cheese using different DHS methods.

	Method A ^a	Method B ^b	Method C ^b
ethanoic acid	1.48 \pm 0.39 ^a	1.78 \pm 0.90 ^a	0.01 \pm 0.00 ^b
<i>n</i> -butanoic acid	65.55 \pm 5.78 ^a	1.62 \pm 0.66 ^b	0.03 \pm 0.01 ^b
<i>n</i> -acids	6.17 \pm 0.91 ^a	0.20 \pm 0.12 ^b	0.01 \pm 0.00 ^b
branched-chain acids	17.06 \pm 5.34 ^a	2.03 \pm 0.71 ^b	0.05 \pm 0.02 ^b
Total acids	90.27 \pm 2.47^a	5.62 \pm 2.39^b	0.10 \pm 0.03^c
ethanol	ND ^a	8.19 \pm 0.82 ^b	2.23 \pm 0.46 ^c
2-butanol	1.88 \pm 1.04 ^a	66.81 \pm 2.91 ^b	81.52 \pm 1.15 ^c
primary alcohols	2.81 \pm 0.63 ^a	7.21 \pm 0.91 ^b	5.93 \pm 0.43 ^b
secondary alcohols	0.18 \pm 0.07 ^a	1.55 \pm 0.31 ^b	1.80 \pm 0.16 ^b
branched-chain alcohols	0.10 \pm 0.05 ^a	0.18 \pm 0.02 ^a	0.36 \pm 0.05 ^b
oxy ether alcohols	0.72 \pm 0.27 ^a	0.05 \pm 0.00 ^b	0.07 \pm 0.01 ^b
aromatic alcohols	ND ^a	ND ^a	ND ^a
Total alcohols	5.70 \pm 1.20^a	83.99 \pm 3.31^b	91.90 \pm 1.27^c
<i>n</i> -aldehydes	0.76 \pm 0.25 ^a	0.11 \pm 0.01 ^b	0.08 \pm 0.01 ^b
branched-chain aldehydes	ND ^a	0.06 \pm 0.02 ^b	0.11 \pm 0.03 ^c
aromatic aldehydes	ND ^a	ND ^a	ND ^a
Total aldehydes	0.76 \pm 0.25^a	0.16 \pm 0.02^b	0.19 \pm 0.02^b
methyl ketones	0.08 \pm 0.04 ^a	8.71 \pm 1.31 ^b	4.73 \pm 1.38 ^c
propyl ketones	ND ^a	ND ^a	ND ^a
aromatic ketones	ND ^a	ND ^a	< 0.01 ^b
diketones	ND ^a	0.05 \pm 0.10 ^b	0.08 \pm 0.01 ^b
Total ketones	0.08 \pm 0.04^a	8.77 \pm 1.31^b	4.82 \pm 1.40^c
methyl esters	ND ^a	ND ^a	0.01 \pm 0.00 ^b
ethyl esters	0.44 \pm 0.17 ^a	0.17 \pm 0.02 ^a	0.95 \pm 0.02 ^b
propyl esters	0.10 \pm 0.10 ^a	0.02 \pm 0.00 ^a	0.58 \pm 0.04 ^b
butyl esters	0.50 \pm 0.07 ^a	0.04 \pm 0.01 ^b	0.13 \pm 0.03 ^c
higher alkyl esters	0.37 \pm 0.50 ^a	ND ^b	ND ^b
branched alkyl esters	1.10 \pm 0.63 ^a	0.29 \pm 0.02 ^b	0.90 \pm 0.08 ^{ab}
Total esters	2.50 \pm 1.20^a	0.52 \pm 0.04^b	2.57 \pm 0.16^a
alkanes	0.67 \pm 0.57 ^{ab}	0.84 \pm 0.11 ^a	0.06 \pm 0.00 ^b
aromatic hydrocarbons	0.04 \pm 0.01 ^a	0.01 \pm 0.01 ^a	0.23 \pm 0.13 ^b
unsaturated hydrocarbons	ND ^a	0.06 \pm 0.01 ^b	0.09 \pm 0.00 ^c
Total hydrocarbons	0.70 \pm 0.57^a	0.91 \pm 0.11^a	0.38 \pm 0.13^a
sulphur compounds	ND ^a	0.03 \pm 0.01 ^b	0.03 \pm 0.10 ^b
terpenes	ND ^a	ND ^a	0.01 \pm 0.01 ^b

^a Method using P&T-MWH. ^b Methods using P&T-RH. ND: not detected.

^{a-c} Means within rows without a common superscript are significantly different ($P \leq 0.05$).

Table IV. Percentage (means \pm SD; $n = 4$) of the volatile compounds found in Roncal cheese using different DHS methods.

	Method A ^a	Method B ^b	Method C ^b
ethanoic acid	3.17 \pm 0.64 ^a	0.43 \pm 0.13 ^b	< 0.01 ^b
<i>n</i> -butanoic acid	37.35 \pm 2.47 ^a	0.67 \pm 0.05 ^b	0.01 \pm 0.00 ^b
<i>n</i> -acids	32.27 \pm 1.07 ^a	0.06 \pm 0.02 ^b	ND ^b
branched-chain acids	7.82 \pm 3.51 ^a	1.78 \pm 0.83 ^b	< 0.01 ^b
Total acids	80.62 \pm 0.96^a	2.93 \pm 0.76^b	0.01 \pm 0.00^c
ethanol	ND ^a	11.01 \pm 1.66 ^b	3.33 \pm 0.55 ^c
2-butanol	9.06 \pm 1.18 ^a	53.28 \pm 2.66 ^b	73.97 \pm 1.81 ^c
primary alcohols	1.52 \pm 0.39 ^a	5.60 \pm 0.61 ^b	4.47 \pm 0.55 ^c
secondary alcohols	ND ^a	1.26 \pm 0.27 ^b	1.34 \pm 0.14 ^b
branched-chain alcohols	0.71 \pm 0.45 ^a	0.170 \pm 0.16 ^a	0.18 \pm 0.02 ^a
oxy ether alcohols	0.52 \pm 0.24 ^a	0.06 \pm 0.06 ^b	0.02 \pm 0.00 ^b
aromatic alcohols	ND ^a	0.14 \pm 0.26 ^b	ND ^a
Total alcohols	11.80 \pm 1.62^a	71.52 \pm 2.77^b	83.30 \pm 2.79^c
<i>n</i> -aldehydes	1.26 \pm 0.50 ^a	0.10 \pm 0.02 ^b	0.19 \pm 0.02 ^b
branched-chain aldehydes	ND ^a	0.08 \pm 0.06 ^b	0.15 \pm 0.02 ^b
aromatic aldehydes	ND ^a	ND ^a	0.01 \pm 0.00 ^b
Total aldehydes	1.26 \pm 0.50^a	0.18 \pm 0.08^b	0.35 \pm 0.05^b
methyl ketones	1.28 \pm 0.56 ^a	23.68 \pm 3.93 ^b	15.21 \pm 2.71 ^c
propyl ketones	ND ^a	ND ^a	ND ^a
aromatic ketones	ND ^a	ND ^a	< 0.01 ^b
diketones	ND ^a	0.32 \pm 0.23 ^b	0.17 \pm 0.05 ^b
Total ketones	1.28 \pm 0.56^a	24.00 \pm 3.86^b	15.38 \pm 2.76^c
methyl esters	ND ^a	0.02 \pm 0.01 ^b	0.01 \pm 0.01 ^b
ethyl esters	0.47 \pm 0.30 ^a	0.11 \pm 0.03 ^b	0.26 \pm 0.03 ^{ab}
propyl esters	ND ^a	ND ^a	0.14 \pm 0.03 ^b
butyl esters	0.45 \pm 0.16 ^a	ND ^b	0.01 \pm 0.00 ^c
higher alkyl esters	ND ^a	ND ^a	ND ^a
branched alkyl esters	0.37 \pm 0.10 ^a	0.06 \pm 0.02 ^b	0.11 \pm 0.03 ^b
Total esters	1.29 \pm 0.26^a	0.19 \pm 0.02^b	0.52 \pm 0.10^c
alkanes	3.42 \pm 1.05 ^a	1.09 \pm 0.71 ^b	0.12 \pm 0.06 ^b
aromatic hydrocarbons	0.34 \pm 0.11 ^a	ND ^b	0.21 \pm 0.05 ^a
unsaturated hydrocarbons	ND ^a	0.02 \pm 0.01 ^b	0.04 \pm 0.01 ^c
Total hydrocarbons	3.75 \pm 0.96^a	1.11 \pm 0.71^b	0.37 \pm 0.08^b
sulphur compounds	ND ^a	0.03 \pm 0.02 ^b	0.02 \pm 0.00 ^b
terpenes	ND ^a	0.03 \pm 0.01 ^b	0.05 \pm 0.01 ^c

^a Method using P&T-MWH. ^b Methods using P&T-RH. ND: not detected.

^{a-c} Means within rows without a common superscript are significantly different ($P \leq 0.05$).

Table V. Percentage (means \pm SD; $n = 4$) of the volatile compounds found in Zamorano cheese using different DHS methods.

	Method A ^a	Method B ^b	Method C ^b
ethanoic acid	33.75 \pm 8.25 ^a	3.08 \pm 0.74 ^b	0.02 \pm 0.00 ^b
<i>n</i> -butanoic acid	31.74 \pm 7.61 ^a	2.23 \pm 0.39 ^b	0.02 \pm 0.00 ^b
<i>n</i> -acids	8.03 \pm 2.07 ^a	0.27 \pm 0.05 ^b	ND ^c
branched-chain acids	4.36 \pm 0.88 ^a	2.17 \pm 0.35 ^b	ND ^c
Total acids	77.88 \pm 6.62^a	7.74 \pm 1.49^b	0.04 \pm 0.01^c
ethanol	ND ^a	28.25 \pm 2.08 ^b	12.36 \pm 0.60 ^c
2-butanol	8.15 \pm 3.26 ^a	35.74 \pm 3.04 ^b	56.36 \pm 6.67 ^c
primary alcohols	0.80 \pm 0.06 ^a	2.80 \pm 0.36 ^b	1.69 \pm 0.54 ^c
secondary alcohols	2.99 \pm 1.70 ^a	4.36 \pm 0.51 ^{ab}	5.62 \pm 0.50 ^b
branched-chain alcohols	2.07 \pm 1.63 ^a	0.28 \pm 0.03 ^b	0.62 \pm 0.07 ^{ab}
oxy ether alcohols	0.49 \pm 0.19 ^a	0.05 \pm 0.00 ^b	ND ^b
aromatic alcohols	ND ^a	ND ^a	ND ^a
Total alcohols	14.49 \pm 5.56^a	71.48 \pm 3.18^b	76.65 \pm 6.44^b
<i>n</i> -aldehydes	2.53 \pm 0.46 ^a	0.27 \pm 0.02 ^b	0.32 \pm 0.10 ^b
branched-chain aldehydes	ND ^a	0.19 \pm 0.07 ^b	0.37 \pm 0.4 ^c
aromatic aldehydes	ND ^a	ND ^a	0.20 \pm 0.01 ^b
Total aldehydes	2.53 \pm 0.46^a	0.46 \pm 0.09^b	0.71 \pm 0.14^b
methyl ketones	2.54 \pm 0.59 ^a	16.02 \pm 2.44 ^b	12.57 \pm 1.96 ^b
propyl ketones	ND ^a	ND ^a	ND ^a
aromatic ketones	ND ^a	ND ^a	0.01 \pm 0.00 ^b
diketones	ND ^a	0.43 \pm 0.15 ^b	0.89 \pm 0.06 ^c
Total ketones	2.54 \pm 0.59^a	16.45 \pm 2.55^b	13.46 \pm 2.01^b
methyl esters	ND ^a	0.07 \pm 0.01 ^b	0.08 \pm 0.04 ^b
ethyl esters	1.76 \pm 0.75 ^a	0.39 \pm 0.05 ^b	0.73 \pm 0.12 ^b
propyl esters	ND ^a	ND ^a	0.03 \pm 0.00 ^b
butyl esters	ND ^a	ND ^a	0.02 \pm 0.01 ^b
higher alkyl esters	ND ^a	ND ^a	ND ^a
branched alkyl esters	0.21 \pm 0.08 ^a	0.08 \pm 0.01 ^b	0.09 \pm 0.03 ^b
Total esters	0.96 \pm 0.81^a	0.54 \pm 0.06^b	0.96 \pm 0.08^b
alkanes	0.60 \pm 0.18 ^a	2.42 \pm 0.76 ^a	7.37 \pm 8.43 ^a
aromatic hydrocarbons	ND ^a	0.01 \pm 0.00 ^b	0.58 \pm 0.13 ^c
unsaturated hydrocarbons	ND ^a	0.08 \pm 0.02 ^b	0.10 \pm 0.01 ^b
Total hydrocarbons	0.60 \pm 0.18^a	2.52 \pm 0.75^a	8.05 \pm 8.30^a
sulphur compounds	ND ^a	0.82 \pm 0.25 ^b	0.09 \pm 0.01 ^c
terpenes	ND ^a	ND ^a	0.03 \pm 0.01 ^b

^a Method using P&T-MWH. ^b Methods using P&T-RH. ND: not detected.^{a-c} Means within rows without a common superscript are significantly different ($P \leq 0.05$).

Table VI. Percentage (means \pm SD; $n = 4$) of the volatile compounds found in Manchego using different DHS methods.

	Method A ^a	Method B ^b	Method C ^b
ethanoic acid	1.46 \pm 0.94 ^a	1.01 \pm 0.59 ^a	< 0.01 ^b
<i>n</i> -butanoic acid	5.50 \pm 4.50 ^a	0.84 \pm 0.43 ^{ab}	0.01 \pm 0.00 ^b
<i>n</i> -acids	ND ^a	0.11 \pm 0.04 ^b	ND ^a
branched-chain acids	2.4 \pm 2.16 ^a	0.93 \pm 0.49 ^a	< 0.01 ^a
Total acids	9.37 \pm 5.70^a	2.88 \pm 1.55^{ab}	0.01 \pm 0.00^b
ethanol	ND ^a	12.07 \pm 0.80 ^b	3.57 \pm 0.42 ^c
2-butanol	48.90 \pm 22.72 ^a	59.61 \pm 3.40 ^{ab}	80.36 \pm 0.65 ^b
primary alcohols	7.98 \pm 3.94 ^a	5.22 \pm 0.80 ^a	4.35 \pm 0.29 ^a
secondary alcohols	3.14 \pm 1.72 ^a	4.84 \pm 0.50 ^{ab}	5.61 \pm 0.42 ^b
branched-chain alcohols	0.77 \pm 0.41 ^a	0.13 \pm 0.02 ^b	0.30 \pm 0.01 ^{ab}
oxy ether alcohols	0.89 \pm 0.82 ^a	0.02 \pm 0.01 ^a	0.05 \pm 0.01 ^a
aromatic alcohols	ND ^a	ND ^a	ND ^a
Total alcohols	61.68 \pm 19.25^a	81.88 \pm 2.40^{ab}	94.25 \pm 0.45^b
<i>n</i> -aldehydes	3.00 \pm 1.97 ^a	0.12 \pm 0.02 ^b	0.08 \pm 0.02 ^b
branched-chain aldehydes	ND ^a	0.08 \pm 0.02 ^b	0.07 \pm 0.00 ^b
aromatic aldehydes	ND ^a	ND ^a	0.01 \pm 0.00 ^b
Total aldehydes	3.00 \pm 1.97^a	0.19 \pm 0.01^b	0.16 \pm 0.01^b
methyl ketones	1.84 \pm 1.65 ^a	14.11 \pm 3.52 ^b	4.19 \pm 0.58 ^a
propyl ketones	0.12 \pm 0.08 ^a	ND ^b	ND ^b
aromatic ketones	ND ^a	ND ^a	< 0.01 ^b
diketones	ND ^a	0.10 \pm 0.04 ^b	0.05 \pm 0.02 ^b
Total ketones	1.96 \pm 1.60^a	14.21 \pm 3.56^b	4.25 \pm 0.60^a
methyl esters	ND ^a	0.02 \pm 0.01 ^b	0.02 \pm 0.01 ^b
ethyl esters	2.43 \pm 1.68 ^a	0.08 \pm 0.01 ^b	0.22 \pm 0.02 ^b
propyl esters	0.21 \pm 0.14 ^a	ND ^b	0.08 \pm 0.01 ^a
butyl esters	ND ^a	ND ^a	0.03 \pm 0.00 ^b
higher alkyl esters	ND ^a	ND ^a	ND ^a
branched alkyl esters	1.82 \pm 0.85 ^a	0.06 \pm 0.01 ^b	0.17 \pm 0.03 ^b
Total esters	4.46 \pm 2.27^a	0.17 \pm 0.03^b	0.52 \pm 0.01^b
alkanes	0.72 \pm 0.42 ^a	0.43 \pm 0.28 ^a	0.46 \pm 0.69 ^a
aromatic hydrocarbons	0.53 \pm 0.31 ^a	0.01 \pm 0.00 ^b	0.22 \pm 0.12 ^b
unsaturated hydrocarbons	16.27 \pm 15.38 ^a	0.01 \pm 0.00 ^b	0.01 \pm 0.00 ^b
Total hydrocarbons	17.52 \pm 15.73^a	0.44 \pm 0.28^b	0.69 \pm 0.62^b
sulphur compounds	ND ^a	0.15 \pm 0.15 ^b	0.02 \pm 0.01 ^b
terpenes	2.02 \pm 0.84 ^a	0.08 \pm 0.01 ^b	0.11 \pm 0.01 ^b

^a Method using P&T-MWH. ^b Methods using P&T-RH. ND: not detected.

^{a-c} Means within rows without a common superscript are significantly different ($P \leq 0.05$).

Table VII. Percentage (means \pm SD; $n = 4$) of the volatile compounds found in La Serena cheese using different DHS methods.

	Method A ^a	Method B ^b	Method C ^b
ethanoic acid	ND ^a	0.56 \pm 0.29 ^b	ND ^a
<i>n</i> -butanoic acid	5.55 \pm 1.60 ^a	0.73 \pm 0.37 ^b	0.01 \pm 0.01 ^b
<i>n</i> -acids	ND ^a	0.08 \pm 0.04 ^b	ND ^a
branched-chain acids	ND ^a	1.05 \pm 0.52 ^b	ND ^a
Total acids	5.55 \pm 1.60^a	2.41 \pm 1.18^b	0.01 \pm 0.01^c
ethanol	ND ^a	67.05 \pm 12.28 ^b	51.40 \pm 1.82 ^c
2-butanol	ND ^a	5.49 \pm 3.52 ^b	3.86 \pm 3.12 ^b
primary alcohols	7.40 \pm 2.52 ^{ab}	5.78 \pm 1.61 ^a	10.00 \pm 0.68 ^b
secondary alcohols	1.30 \pm 0.23 ^a	1.62 \pm 0.24 ^a	1.26 \pm 0.67 ^a
branched-chain alcohols	6.36 \pm 4.77 ^a	4.56 \pm 1.31 ^a	18.03 \pm 1.03 ^b
oxy ether alcohols	ND ^a	0.09 \pm 0.02 ^b	0.28 \pm 0.04 ^c
aromatic alcohols	ND ^a	0.01 \pm 0.01 ^b	0.02 \pm 0.01 ^b
Total alcohols	15.06 \pm 3.88^a	84.58 \pm 6.44^b	84.81 \pm 3.05^b
<i>n</i> -aldehydes	58.39 \pm 12.14 ^a	0.35 \pm 0.09 ^b	0.52 \pm 0.11 ^b
branched-chain aldehydes	ND ^a	0.21 \pm 0.13 ^b	0.54 \pm 0.13 ^c
aromatic aldehydes	ND ^a	ND ^a	ND ^a
Total aldehydes	58.39 \pm 12.14^a	0.56 \pm 0.06^b	1.06 \pm 0.24^b
methyl ketones	3.22 \pm 2.08 ^a	7.96 \pm 5.11 ^a	1.71 \pm 1.68 ^a
propyl ketones	ND ^a	ND ^a	ND ^a
aromatic ketones	1.29 \pm 0.90 ^a	ND ^b	0.03 \pm 0.00 ^c
diketones	ND ^a	0.18 \pm 0.13 ^b	0.61 \pm 0.19 ^c
Total ketones	4.51 \pm 2.86^a	8.13 \pm 5.22^a	2.35 \pm 1.86^a
methyl esters	ND ^a	ND ^a	< 0.01 ^b
ethyl esters	13.34 \pm 9.71 ^a	2.94 \pm 0.50 ^a	10.90 \pm 4.70 ^a
propyl esters	ND ^a	ND ^a	0.03 \pm 0.02 ^b
butyl esters	ND ^a	ND ^a	0.04 \pm 0.04 ^b
higher alkyl esters	ND ^a	0.01 \pm 0.00 ^b	ND ^a
branched alkyl esters	0.87 \pm 0.80 ^a	0.11 \pm 0.03 ^a	0.40 \pm 0.16 ^a
Total esters	14.20 \pm 10.17^a	3.05 \pm 0.53^a	11.38 \pm 4.87^a
alkanes	1.92 \pm 1.07 ^a	1.18 \pm 0.24 ^{ab}	0.20 \pm 0.07 ^b
aromatic hydrocarbons	0.37 \pm 0.19 ^a	ND ^b	0.09 \pm 0.04 ^c
unsaturated hydrocarbons	ND ^a	0.01 \pm 0.01 ^b	0.02 \pm 0.00 ^b
Total hydrocarbons	2.29 \pm 1.20^a	1.19 \pm 0.24^{ab}	0.31 \pm 0.10^b
sulphur compounds	ND ^a	0.07 \pm 0.01 ^b	0.04 \pm 0.04 ^b
terpenes	ND ^a	ND ^a	0.04 \pm 0.01 ^b

^a Method using P&T-MWH. ^b Methods using P&T-RH. ND: not detected.^{a-c} Means within rows without a common superscript are significantly different ($P \leq 0.05$).

three methods were rather similar (Tabs. III–VII).

The above mentioned results were partially in accordance with another study in which P&T-RH was compared with P&T-MWH for the analysis of the volatile compounds of Swiss Emmental cheese [5]. The authors concluded that P&T-RH was the most efficient for the determination of alcohols, carbonyl compounds, esters, hydrocarbons and sulphur compounds.

Considerable differences were also recorded in the percentages of the volatile compounds found in the PDO cheeses between the methods **B** and **C** using P&T-RH under different analytical conditions. A larger purge volume, higher temperatures, and higher proportion of Na_2SO_4 added to the cheese sample were used in method **C** to carry out the DHS of cheese volatiles, compared with method **B**. In general, larger percentages of acids and ketones were found by method **B** compared with method **C** in all the PDO cheese varieties, whereas the contrary occurred with the percentages of esters (Tabs. III–VII). Non-significant ($P > 0.05$) differences were found between the two methods for the percentages of alcohols in Zamorano, Manchego and La Serena cheeses, but larger percentages of alcohols, particularly 2-butanol, were found by method **C** compared with method **B** in Idiazabal and Roncal cheeses. Non-significant ($P > 0.05$) differences were also recorded for the percentages of other volatile compounds such as aldehydes, hydrocarbons and sulphur compounds in all of the PDO cheese varieties (Tables III–VII). Similar results were obtained by other authors in the analysis of the volatile compounds of Manchego, Roncal, Zamorano and La Serena cheeses by DHS using P&T-RH [7, 15, 16, 25].

The coefficients of variation for the percentages of the volatile compounds found in all of the PDO cheese varieties by each of the three methods were, in general, higher than 10%. However, the methods **B** and **C** which used automatic P&T-RH showed lower coefficients of variation for most chemical families than those of the method **A**, which used semi-automatic P&T-MWH. The mean value for the coefficients of variation for all the chemical

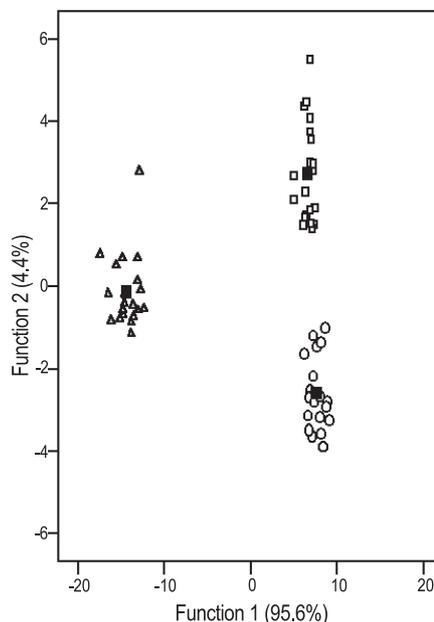


Figure 1. Cheese sample distribution according to DHS method used for volatile compound analysis in the two-dimensional coordinate system defined by canonical discriminant functions. (triangle): method **A** using P&T-MWH; (circle): method **B** using P&T-RH; (square): method **C** using P&T-RH.

families obtained by method **A** was 45.2%, whereas 26.5 and 21.8% were the mean values obtained by methods **B** and **C**, respectively. These results confirmed better repeatability for the DHS methods which used automatic P&T-RH than for the DHS method which used semi-automatic P&T-MWH [23].

Stepwise discriminant analysis was applied to classify the cheese samples according to the DHS method used. The samples of the five PDO cheeses were correctly classified according to the method. Function 1, which accounted for 95.6% of variance, distinguished between method **A** and methods **B** and **C**, whereas function 2, which accounted for only 4.4% of variance, distinguished between method **B** and method **C** (Fig. 1). Volatile compounds

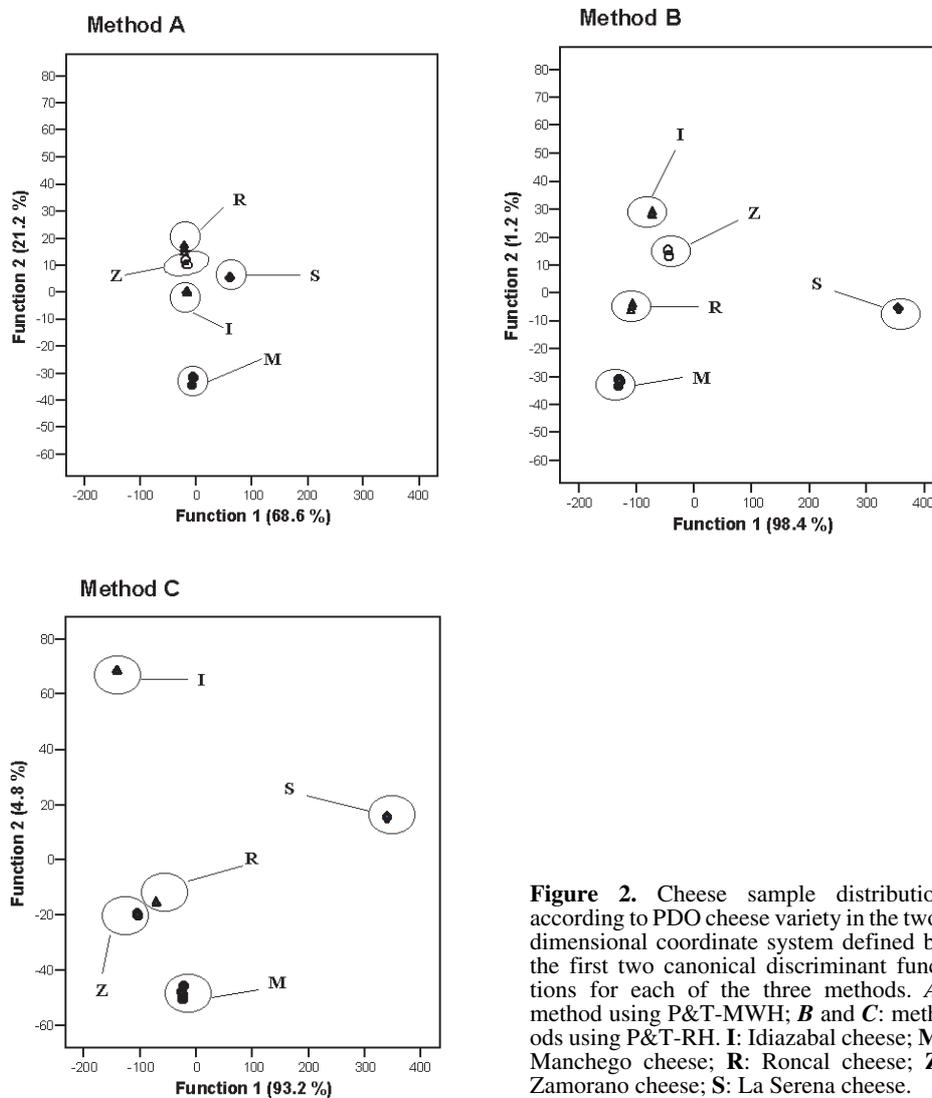


Figure 2. Cheese sample distribution according to PDO cheese variety in the two-dimensional coordinate system defined by the first two canonical discriminant functions for each of the three methods. **A:** method using P&T-MWH; **B** and **C:** methods using P&T-RH. **I:** Idiazabal cheese; **M:** Manchego cheese; **R:** Roncal cheese; **Z:** Zamorano cheese; **S:** La Serena cheese.

such as sulphur compounds, total and secondary alcohols, methyl esters, *n*-butanoic acid and unsaturated hydrocarbons were closely correlated with function 1, whereas straight, aromatic and total aldehydes, propyl esters, methyl ketones and aromatic hydrocarbons were closely correlated with function 2. These results showed clearly that, in this particular matter (volatile composition research), the results obtained by

different methods must be carefully compared, even if the same cheese piece is analysed by different laboratories using similar DHS systems.

Stepwise discriminant analyses were also applied separately to classify the PDO cheese varieties according to the volatile composition obtained by each of the three methods. As observed in Figure 2, the samples were correctly classified according to

Table VIII. Discriminant variables included in the stepwise discriminant analyses applied to classify the PDO cheese varieties by different DHS methods.

Method <i>A</i> ^a	Method <i>B</i> ^b	Method <i>C</i> ^b
secondary alcohols	secondary alcohols	secondary alcohols
ethyl esters	ethyl esters	propyl esters
<i>n</i> -aldehydes	branched-chain alcohols	branched-chain alcohols
<i>n</i> -acids	aromatic alcohols	aromatic alcohols
ethanoic acid	ethanol	ethanol
total acids	terpenes	oxy ether alcohols
propyl ketones	branched alkyl esters	aromatic aldehydes
aromatic ketones	higher alkyl esters	total ketones
	aromatic hydrocarbons	branched-chain acids
		unsaturated hydrocarbons

^a Method using P&T-MWH. ^b Methods using P&T-RH.

the cheese PDO variety in each of the three methods. Function 1, accounting for 68.6, 98.4 and 93.2% of variance, respectively, for methods *A*, *B* and *C*, distinguished between La Serena cheese and the other four PDO cheese varieties. These results pointed out that the largest differences were recorded between the soft cheese variety made with vegetable rennet and the other semi-hard cheese varieties made with animal rennet. Function 2, accounting for 21.2, 1.2 and 4.8% of variance, respectively, for methods *A*, *B* and *C*, classified correctly the four semi-hard cheese varieties (Fig. 2). Although the three methods were capable of distinguishing between the volatile composition of the five PDO cheese varieties, the distances from each other for group centroids of each type of cheese were larger for methods *B* and *C* using automatic P&T-RH than for method *A* using semi-automatic P&T-MWH (Fig. 2). Table VIII shows the discriminant variables used to classify the PDO cheeses in each of the three methods. In general, different volatile compounds were used as discriminant variables for each of the three methods. It was remarkable that the secondary alcohols group was the only discriminant variable common to the three methods, and that different alcohol compounds were common to the methods which used automatic P&T-RH (Tab. VIII). For method *A*, volatile compounds such as

n-acids, total acids, ethanoic acid, *n*-aldehydes or ethyl esters, whose percentages showed large differences among the PDO cheeses, were used as discriminant variables. For methods *B* and *C*, volatile compounds such as ethanol, branched-chain alcohols and total ketones, whose percentages showed large differences among the PDO cheeses, were used as discriminant variables.

In summary, the results pointed out differences in the extraction of volatile compounds from the cheese samples according to the different DHS system used, that were semi-automatic P&T-MWH and automatic P&T-RH. The nature of the trap adsorbent, i.e. graphitised carbon for P&T-MWH and tenax for P&T-RH, was determinant for the selectivity of the adsorption of cheese volatiles. Differences in the extraction of volatile compounds were also observed between laboratories which used the same DHS system under different analytical conditions, i.e. different automatic sampler model, sample preparation, and conditions for purge, concentration on trap, and thermal desorption.

For the comparison of different cheese varieties manufactured in the same period, the DHS methods used in this study are equally satisfactory as shown by the correct classification of the 4 samples from 2 batches of each cheese variety by the discriminant analyses. However, the results on

volatile composition obtained by different methods must be carefully compared, even if the same cheese piece is analysed using similar DHS systems under different analytical conditions. The P&T-MWH and P&T-RH systems assayed in this work have advantages and disadvantages for the exhaustive characterisation of the aroma compounds of a particular cheese. For the largest lipolysed cheeses such as Idiazabal, Roncal and Zamorano, P&T-MWH provides only a relatively poor aroma profile which is mainly composed of volatile acids, whereas P&T-RH gives more information on other aroma compounds such as methyl ketones. For the lowest lipolysed cheeses such as Manchego and La Serena, P&T-RH provides a mainly alcoholic profile, whereas P&T-MWH gives more information on other aroma components such as volatile acids and aldehydes.

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