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Composition of the Water-Soluble Fraction of Different Cheeses

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Volatile and nonvolatile compounds present in the water-soluble fraction (WSF) and water-soluble fraction with molecular weight lower than 1000 Da (WSF < 1000 Da) of six Spanish cheeses, Cabrales, Idiazábal, Mahón, Manchego, Roncal, and a goat's milk cheese, were analyzed. Different nitrogen fractions (determined by Kjeldahl method), caseins (by capillary electrophoresis), peptides and amino acids (by HPLC), and volatile components (by dynamic headspace coupled to GC-MS) as well as mineral content in the cheese fractions were analyzed and compared. The different nitrogen and volatile compounds identified in the WSF were characteristic of each cheese variety. Cabrales cheese displayed the highest content of free amino acids and the highest quantity and variety of volatile compounds. The WSF < 1000 Da fraction was less representative, especially for volatile compounds, as some of the components were lost in the ultrafiltration. Alcohols were better recovered than ketones and esters.

KEYWORDS: Water-soluble nitrogen; volatile compounds; Spanish cheeses

INTRODUCTION

The Spanish cheese sector is characterized by a long cheese-making tradition and by a wide diversity of cheeses (81 referenced types) with very peculiar sensorial characteristics. Most of these traditional cheeses are well established and duly described, and several possess Protected Denomination of Origin (PDO) such as Cabrales, Idiazábal, Mahón, Roncal, and Manchego cheeses among others. Many studies have contributed to our knowlege of the composition of cheeses, the biochemical reactions that take place during ripening, and the microbial ecology in several varieties of Spanish cheeses exploited to improve their microbiological quality and to obtain well-defined final cheeses (*1*, *2*).

The water-soluble fraction (WSF) of cheeses contains the components that make a major contribution to flavor (*3*). This fraction contains amino acids, peptides, mineral salts, lactic acid, lactose, and volatile compounds. Although a part of the volatile compounds is dissolved in fat, or conjugated with insoluble substances, it seems that those present in the WSF could play an important role in the cheese flavor. It has been proposed that the intense aroma of goat's milk cheese is mainly due to specific polar volatile compounds contained in the WSF (*4*), although studies about the volatile compounds of the WSF of various types of cheeses from cow's milk have concluded that there is no single compound or class of compounds responsible for the full flavor of cheeses (*5*). On the other hand, nonvolatile compounds, and especially interactions between them, and interactions between salts and amino acids and low molecular weight peptides (*6*), may also make an important contribution to the cheese flavor. However, Engels and Visser (*7*) have shown that the direct contribution of peptides and amino acids to cheese flavor is probably limited, although the conversion of the amino acids to volatile compounds, by incubation of starter cultures, seems to be important as well in the development of cheese flavor (*8*, *9*).

WSF of cheeses made in the same conditions but from milk of different species showed differences in the composition of peptides, amino acids, and salts of this fraction depending on the origin of the milk (*10*). Nevertheless, no work has been conducted on the study of volatile and nonvolatile compounds in the WSF in Spanish cheeses. We have selected six Spanish cheeses representative of the wide variety of cheese in Spain, with characteristic flavors. The compounds present in the WSF (volatiles and nonvolatiles) and especially in the WSF with molecular weight lower than 1000 Da (WSF < 1000 Da) would be the final components that give rise to the typical flavor and taste of each cheese or be precursors of other compounds also involved in the development of the cheese flavor. Therefore, the objective of the present study was to analyze peptides, amino acids, volatiles, and ions in the WSF and WSF < 1000 Da of several PDO Spanish cheeses made with different technologies (mold-ripened, smoked, and hard); the results are related to the peculiar characteristics of each variety.

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MATERIALS AND METHODS

Cheese Samples. Six commercial Spanish cheeses, five of them with PDO, were purchased in a local market. The main characteristics of these types of cheeses have been described by Nuñez et al. (*1*). Cabrales cheese is a blue-veined variety manufactured in northern Spain (Asturias) from mixed cow's, ewe's, and goat's milk, with no starter culture or mold spores added; the cheeses are then ripened in caves under natural air currents. Idiázabal cheese is a hard variety from northern Spain (Basque country) made with ewe's milk. After the first month of ripening, the cheese is treated with the smoke from burning beech wood. Mahón cheese is produced in Menorca (Balearic Islands) with cow's milk. When pasteurized milk is used, it is inoculated with lactic starter. Manchego cheese is perhaps the most popular cheese variety in Spain. It is a hard or semihard variety made in central Spain (La Mancha) from ewe's milk. Roncal cheese is a hard cheese manufactured in northern Spain from ewe's milk, with a technology similar to that of Manchego cheese. Usually, these cheeses are made from raw milk if they are produced artisanally and from pasteurized milk if they are industrially produced.

In this study, all of the cheeses were made from pasteurized milk except Manchego cheese, and the regular and most frequent ages of consumption were chosen. Cabrales, Idiazábal, Manchego, and Roncal cheeses were semicured cheeses (around 4 months old); goat's milk cheese was around 2 months old, and Mahón cheese was used after <2 months of ripening.

Preparation of Water-Soluble Fraction and Caseins. The WSF was prepared following the method of Salles et al. (*3*)**.** The WSF was passed through a membrane with a molecular weight cutoff of 1000 Da to obtain the WSF < 1000 Da (YM1, DIAFLO-Filtron Technology Corp., Northborough, MA) at 5 °C in a stirred-cell type ultrafiltration module (υ = 50 mL, i.d. = 43 mm) (Amicon, Danvers, MA), under a pressure of 45 psi applied with nitrogen.

Caseins were obtained from 5 g of cheese homogenized with 8 mL of Milli-Q water (Millipore Corp.). The pH of the homogenates was adjusted with 2 M HCl at pH 4.6, held for 2 h, and centrifuged at 4000*g* and 5 °C for 20 min. The casein precipitate was washed and lyophilized. All of the samples were obtained and analyzed in duplicate.

Chemical Analysis. Dry matter (DM) of cheeses was determined according to the IDF standard (*11*). Total nitrogen (TN), water-soluble nitrogen (WSN), and water-soluble nitrogen in the fraction with molecular weight lower than 1000 Da (WSN < 1000 Da) were determined according to the Kjeldahl method as described in IDF standard 20B (*12*). Total nitrogen was expressed as percentage(s) of DM.

Inorganic phosphorus, chloride, and calcium ions were estimated by diagnostic kits (Sigma Chemical Co., St. Louis, MO) in a UV-120-01 spectrophotometer (Shimadzu Corp., Kyoto, Japan) at 340, 460, and 660 nm, respectively.

Capillary Electrophoresis (CE). CE was carried out using a Beckman P/ACE System MDQ controlled by its software data system (Beckman Instruments Inc., Fullerton, CA). Caseins were separated using a hydrophilically coated fused-silica capillary column CElect P1 (Supelco, Bellefonte, PA) of 0.50 μm i.d. and identified following the method of Molina et al. (*13*).

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC). Analyses of peptides and free amino acids were carried out by HPLC. Separation of peptides was performed following the method described by González de Llano et al. (*14*). A Beckman System Gold HPLC equipped with a diode array detector M168 was used together with System Gold software data version 711 (Beckman Instruments Inc.). Separations were performed on a C18 Nova Pak, 4 μm, 60 Å column (150 mm × 3.9 mm) (Waters Corp., Milford, MA), at room temperature. Commercial standards of tryptophan (Trp), phenylalanine (Phe), and tyrosine (Tyr) (Sigma Chemical Co.) were used to make a partial identification of these residues in the samples of cheeses, following the procedure of Bartolomé et al. (*15*) based on the spectral characteristics of those aromatic amino acids. The retention time of Trp was used to differentiate the hydrophobic and hydrophilic peptides.

Analyses of free amino acids were carried out by HPLC of their *o*-phthaldehyde (OPA) derivatives according to the method described by González de Llano et al. (*16*). Derivative formation was performed automatically, and separations were carried out on a Nova Pak C18 precolumn (Waters Corp.) of 60 Å, 4 μm (20 mm × 3.9 mm) and a Nova Pak C18 column (Waters Corp.) of 60 Å, 4 μm (150 mm × 3.9 mm). Detection was by OPA fluorescence. Quantitative analysis was performed using a calibration curve for each amino acid obtained from a master solution of amino acids (Sigma) to which glutamic acid (Glu), asparagine (Asn), β-alanine (β-Ala), α-alanine (α-Ala), γ-aminobutyric acid, (GABA), tryptophan (Trp), ornithine, and histamine were added.

Table 1. Dry Matter (DM), Total Nitrogen (TN), Water-Soluble Nitrogen (WSN), and Soluble Nitrogen in the Fraction <1000 Da (WSN < 1000 Da) of Different Spanish Cheeses

cheese	DM (g/100 g of cheese)	TN (g/100 g of DM)	WSN/TN (%)	WSN < 1000 Da/ TN (%)	WSN < 1000 Da/ WSN (%)
goat's milk	52.74	5.93	15.01	4.55	30.34
Cabrales	59.52	6.77	38.99	20.09	51.51
Idiazábal	70.96	5.54	22.38	4.15	18.54
Mahón	58.84	6.56	19.21	1.52	7.94
Manchego	63.77	5.43	22.47	4.05	18.03
Roncal	72.56	5.62	16.37	5.69	34.78

Dynamic Headspace and Gas Chromatography—Mass Spectrometry (P&T/GC-MS). Separation and identification of volatile components were performed using a dynamic headspace procedure (P&T/GC-MS) as previously described (*17*), using an automatic purge and trap concentrator 7975A (Hewlett-Packard, Palo Alto, CA) fitted with a cryofocusing unit. The concentrator was coupled to a GC-MS system (HP 5890), equipped with a quadrupole mass detector HP-5971A operating in EI mode at 70 eV. Helium was used as carrier gas through all of the system.

Peaks were identified from bibliographic retention times and mass spectral data from the Wiley library (*18*) and confirmed by using standard compounds when available. Free fatty acids were not eluted in these conditions.

Volatiles content was expressed as peak area counts normalized to the peak area of the internal standard (ethyl pentanoate) and corrected for the ratio ISTD/cheese weight.

RESULTS AND DISCUSSION

Determination of the Nitrogen Contents of the Different Fractions. DM and the content of total nitrogen of the cheeses, WSF and WSF < 1000 Da, are presented in **Table 1**, the results being expressed as percentages of the TN and WSN. These nitrogen fractions are indices of proteolysis, which are different in each variety of cheese and can be used as indicators of the maturation stage. Thus, the percentage of WSN/TN of Cabrales cheese (38.99%) was the highest of all the cheeses studied and possessed the highest ratio of WSN < 1000 Da to WSN as a consequence of the high degree of proteolysis, which can be explained by the high exo and endo peptidase activity from *Penicillium roqueforti* (*2*). Roncal, Manchego, Idiazábal, and Mahón cheeses, in which the milk proteinases from lactic acid bacteria are mainly responsible for the proteolytic degradation, contained similar levels of WSN/TN (accounting for ∼16−22% of the total nitrogen). Mahón cheese, at <2 months of ripening and, therefore, a low level of proteolysis, exhibited much smaller ratios of WSN < 1000 Da to TN and WSN < 1000 Da to WSN than more mature cheese. The goat's milk cheese presented a higher content of WSN < 1000 Da to WSN than expected for a 2-month-old cheese. However, similar levels of nitrogen contents have been found in other goat's milk cheeses (*19*).

Mineral Content. P, Cl, and Ca ion contents of WSF < 1000 Da of cheese samples are given in **Figure 1**. No differences were found with the mineral content of this fraction and the mineral content of the WSF. The highest value corresponded

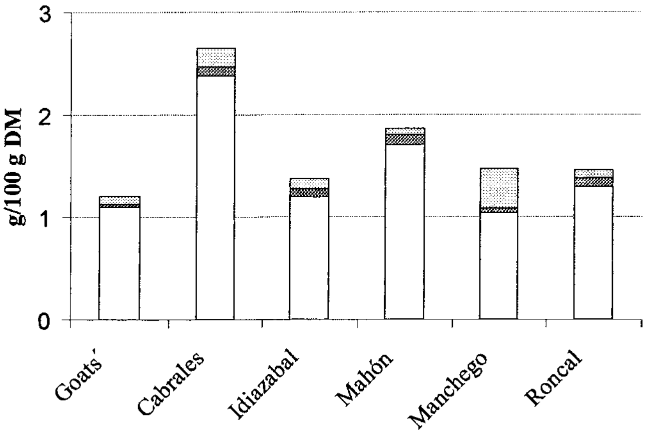

Figure 1. Mineral content: Cl (unshaded bar), Ca (heavily shaded bar), and P (lightly shaded bar) (g/100 g of DM) in the WSF < 1000 Da of goat's milk, Cabrales, Idiazábal, Mahón, Manchego, and Roncal cheeses.

to Cl in all of the cheeses, followed by P and Ca. Cabrales cheese showed the highest value (2.62 g/100 g of DM) for the mineral content, whereas goat's milk cheese (1.21 g/100 g of DM) showed the lowest. The differences found in the mineral composition of the six Spanish cheeses may depend on the conditions of manufacture, coagulation, syneresis, and type of salting. For example, Cabrales cheese presented the highest content of Cl, which was attributed to its dry salting.

This similarity in mineral content of the cheeses studied, which is not in agreement with the diversity of their tastes, is possible because the mineral content is related to the taste of the cheese due to a synergistic effect, essentially with amino acids and low molecular weight peptides (*3*). Nevertheless, sodium chloride has been proved to have a major contribution to the taste profile of the WSE, as reported by Engel et al. (*20*).

Proteolysis of Caseins. Figure 2 shows the capillary electropherograms of the casein fractions of the cheeses in which the different peaks corresponding to caseins from different milk species are well resolved and are used as breakdown indicators. Para-κ-CN f(1−105) arising by rennet action on κ-CN was present in all electropherograms. Bovine and ovine para-κ-CN show the same migration time, which differs from that of caprine para-κ-CN. Para-κ-CN is not proteolyzed during ripening, and it is also resistant to proteases of *P. roqueforti*, which usually impart almost total degradation of the other caseins of the blue cheese (*21*). A peak that corresponds to bovine or ovine para-κ-CN was observed in goat's milk cheese, indicating the presence of milk of species other than goat. α_s-CN with migration times in the intermediate area of the electropherograms are the more degraded proteins in all cheeses. Their proteolysis produces large- and medium-sized peptides that can be subsequently degraded to yield small peptides and free amino acids (FAA). In most of the cheeses it was possible to observe the α_{S1}-I-CN arising from the breakdown of α_{S1}-CN. This casein (α_{S1}-I-CN) was not seen either in Cabrales cheese because of its high degree of proteolysis or in goat's milk cheeses. In the last case it might be attributed to the genetic polymorphism in caprine α_{S1}-CN. Depending on the genetic variants of this protein, its content, the casein content of the milk, and the proportions of the different casein fractions may be different (*22*).

β-CN is known to be the most resistant casein in some ewe's milk cheeses; 90% of intact β-CN has been described at the end of ripening in Manchego cheese (*23*). In this study it is shown that β-CN was largely degraded in Roncal cheeses (**Figure 2f**) and almost totally degraded in Cabrales cheese (**Figure 2b**). There were differences in electrophoretic mobility of β-CN from different species of cheeses. Ovine and caprine β_2-CN had the same migration time as bovine β-CN A^2, and ovine and caprine β_1-CN exhibit the same mobility; however, β-CN A^1 bovine presents a shorter migration time (*13*). These differences could be observed in the capillary electropherogram of casein in Mahón cheese, which showed different genetic variants of bovine β-CN.

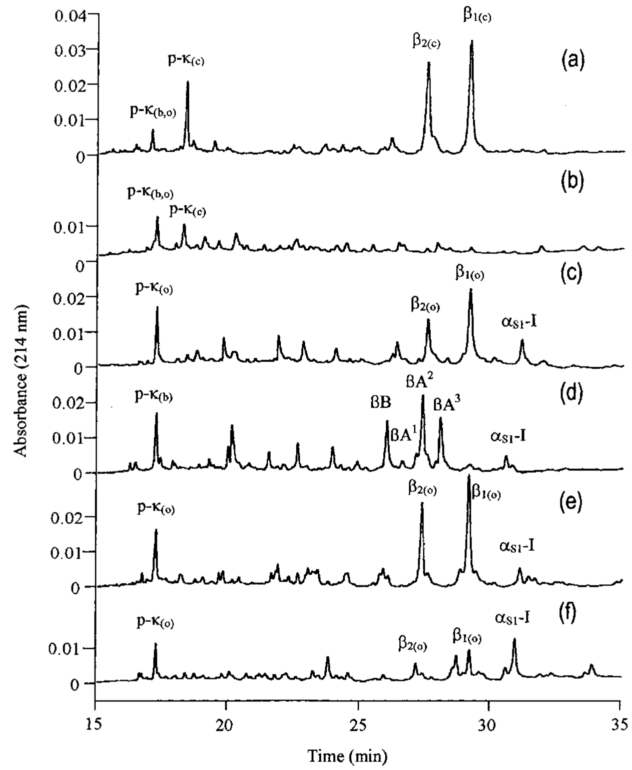

Figure 2. Capillary electropherograms of caseins from goat's milk (a), Cabrales (b), Idiazábal (c), Mahón (d), Manchego (e), and Roncal (f) cheeses. $\beta = \beta$-CN; α_{S1}I = α_{S1}I-CN; p-κ = para-κ-CN; βA = β-CN A; βB = β-CN B; b = bovine; o = ovine; c = caprine.

Analyses of Peptides. The number and concentration of peptides were low except for Cabrales cheese, which was characterized by ~20 peaks (**Figure 3a**). Mahón and goat's milk cheeses had the lowest peptide contents. These results agree with the other proteolysis indices such as WSN and WSN < 1000 Da to NT ratios (**Table 1**). The main peaks of this first eluting hydrophilic component were peptides with Tyr and Phe residues and were mainly found in the WSF < 1000 Da of cheeses studied (**Figure 3b**). In goat's milk cheeses, the high proportion of peaks in the final part of the chromatogram was probably due to the presence of caprine milk, as it has been reported that goats' milk cheeses present a higher proportion of hydrophobic peptides than cheeses from other milk species (*10*). It has been suggested that peptides eluting after Trp (tr = 18 min) constitute the hydrophobic peptide portion, which has been considered to be responsible for the bitter taste in cheeses. Engel et al. (*20*), using a omission test to determine the taste-active compounds of a bitter Camembert cheese, demonstrated that its bitterness arose from small peptides and that it was enhaced by the cheese matrix. However, other studies in the WSF of Bouton-de-Culotte cheese (a French variety made from goat's milk) could not establish the direct relationship between the presence of this kind of peptide to the cheese taste (*6*).

Analyses of Free Amino Acids. Variations in amino acid content of different cheeses were observed. Total FAA correlated

Table 2. Free Amino Acids and Amines (Milligrams per 100 g of DM) in WSF < 1000 Da of Goat's Milk, Cabrales, Idiazábal, Mahón, Manchego, and Roncal Cheeses by RP-HPLC[a]

FAA	goat's milk	Cabrales	Idiazábal	Mahón	Manchego	Roncal
Asp	67.5 (0.3)	158.2 (4.6)	52.3 (2.0)	20.6 (1.3)	35.1 (11.7)	213.9 (5.8)
Glu	137.2 (4.1)	1172.2 (8.2)	326.3 (7.0)	67.7 (2.5)	197.0 (11.4)	665.4 (5.2)
Asn	8.2 (0.7)	112.0 (2.7)	101.1 (4.7)	29.5 (1.8)	34.0 (4.0)	14.4 (0.5)
Ser	25.3 (1.8)	246.4 (3.3)	34.8 (1.0)	14.9 (0.3)	11.8 (2.2)	19.4 (0.3)
Gln	47.1 (4.5)	315.6 (8.0)	78.3 (1.6)	22.8 (1.9)	87.4 (9.7)	51.3 (3.8)
Hys	16.3 (2.1)	154.0 (8.0)	13.1 (4.2)	6.5 (0.3)	37.9 (2.5)	54.5 (4.6)
Gly	21.1 (2.6)	96.1 (8.2)	19.7 (0.8)	15.1 (3.0)	36.8 (5.8)	45.4 (6.8)
Thr	28.0 (0.7)	332.9 (1.1)	87.3 (6.3)	37.3 (1.2)	41.2 (4.4)	63.8 (2.0)
Arg	20.3 (1.8)	51.7 (4.9)	11.8 (2.9)	1.9 (0.1)	93.3 (8.8)	9.6 (1.6)
α-Ala	18.4 (2.1)	569.4 (2.8)	51.1 (5.0)	14.5 (1.3)	89.1 (10.9)	95.0 (0.7)
GABA	0.0	104.9 (0.5)	26.7 (2.8)	0.0	21.3 (1.6)	122.0(3.8)
Tyr	35.4 (5.5)	130.3 (2.4)	74.5 (4.1)	24.4 (0.9)	115.6 (19.5)	23.6 (1.3)
α-Aba	0.0	58.5 (2.1)	0.0	0.0	11.8 (1.6)	15.0 (0.3)
Met	20.7 (2.5)	261.3 (3.5)	51.4 (2.5)	8.3 (1.3)	63.0 (3.9)	74.7 (0.1)
Val	33.3 (2.4)	542.8 (0.1)	184.7 (5.2)	36.3 (1.2)	211.7 (25.9)	239.2 (82.5)
Trp	15.4 (0.9)	82.2 (5.0)	0.0	0.0	0.0	0.0
Phe	41.4 (1.1)	406.9 (11.5)	128.4 (8.0)	75.3 (0.3)	160.8 (9.3)	171.4 (0.3)
Ile	24.0 (1.1)	368.8 (2.5)	62.5 (4.9)	12.9 (0.1)	142.9 (16.6)	154.4 (2.5)
Leu	70.3 (1.0)	756.2 (9.5)	325.3 (13.1)	108.7 (0.2)	334.6 (40.9)	328.3 (1.5)
Lys	59.1 (0.4)	288.7 (9.9)	90.8 (3.71)	40.3 (0.7)	158.3 (15.9)	171.7 (9.1)
Orn	0.0	7.5 (1.6)	3.0 (0.4)	8.9 (0.2)	24.5 (7.7)	9.7 (0.2)
total	688.9	6216.7	1723.1	545.8	1908.2	2542.7
amines						
tyramine	10.6 (0.6)	10.1 (0.3)	3.0 (0.2)	10.2 (0.1)	86.8 (7.0)	7.6 (1.5)

[a]Results are expressed as the mean of two determinations in two different fractionations, standard deviation in parentheses.

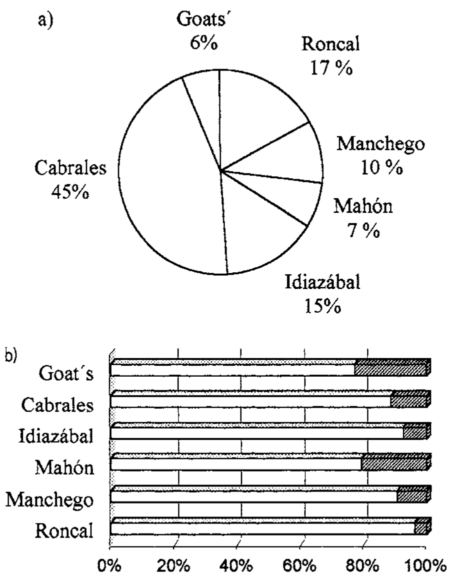

Figure 3. Total peptide content (expressed as the sum of the peak areas) (a) and proportion of hydrophilic to hydrophobic peptides (b) in the WSF < 1000 Da of goat's milk, Cabrales, Idiazábal, Mahón, Manchego, and Roncal cheeses: hydrophobic peptides (shaded bar); hydrophilic peptides (unshaded bar).

with WSN < 1000 Da/NT ($R = 0.965$ for $P < 0.001$). Cabrales cheese had the highest FAA content (6216.7 mg/100 g of DM), whereas Mahón cheese had the lowest (545.8 mg/100 g of DM) (**Table 2**). Ovine cheeses such as Idiazábal, Manchego, and Roncal showed intermediate FAA contents (1723.1, 1908.2, and 2542.7 mg/100 g of DM, respectively). FAA content usually depends on cheese age, although total concentration of amino acids is not directly involved in the development of Cheddar cheese flavor (*24*).

The percent composition of FAA depended on cheese type. Glu and Leu were the predominant free amino acids in all of the cheeses studied; Val and Ile were also abundant in Manchego and Val and Phe in Mahón. Leu has been considered to have an important contribution to cheese flavor in Cheddar cheese (*25*) so, due to its notable presence in all of the cheeses (accounting for 10−20% of the total content in free amino acids), it is also possible it contributes to the flavor of the cheeses studied. Phe was also present in high concentration in Roncal and Idiazábal cheeses. These data are similar to those reported previously in some varieties of cheeses. Barcina et al. (*26*) have reported that Glu, Leu, Val, Lys, Phe, and Ala accounted for 50% of the total FAA content in Idiazábal cheese, and Polo et al. (*27*) found Glu, Val, Phe, Ile, and Pro as the main FAA, which accounted for between 67 and 80% of total FAA in Mahón cheese at 4 months of ripening. Fox and Wallace (*28*) suggested that the flavor and the concentration of free amino acids could not be correlated because different cheeses with the same amino acid composition do not possess the same flavor, but the different catabolism of each amino acid could originate by different pathways, the compounds responsible for the final cheese flavor. Other studies have not found any impact of the amino acids, lactose, and peptides present in goat's milk cheese on the WSE taste properties (*29*).

The absence of γ-aminobutyric acid (GABA) is important in WSF < 1000 Da of goat's milk and Mahón cheeses as is that of α-Aba in goat's milk, Idiazábal, and Mahón cheeses. Trp, which was seen in the peptide analyses, was not detected as FAA in Idiazábal, Mahón, Manchego, and Roncal cheeses, probably due to the small Trp content in those cheeses and poor resolution of the peak, which was a shoulder of the big Val peak (data not shown). Trp is important as its microbiological degradation promotes the formation of compounds with unpleasant flavor; however, the production pathway of such compounds is not established yet (*30*). Ornithine originates from citrulline and is a precursor of putrescine and δ-aminovaleric acid (*31*), but its concentration was not very high, except in Manchego cheese, probably because it was made from raw milk.

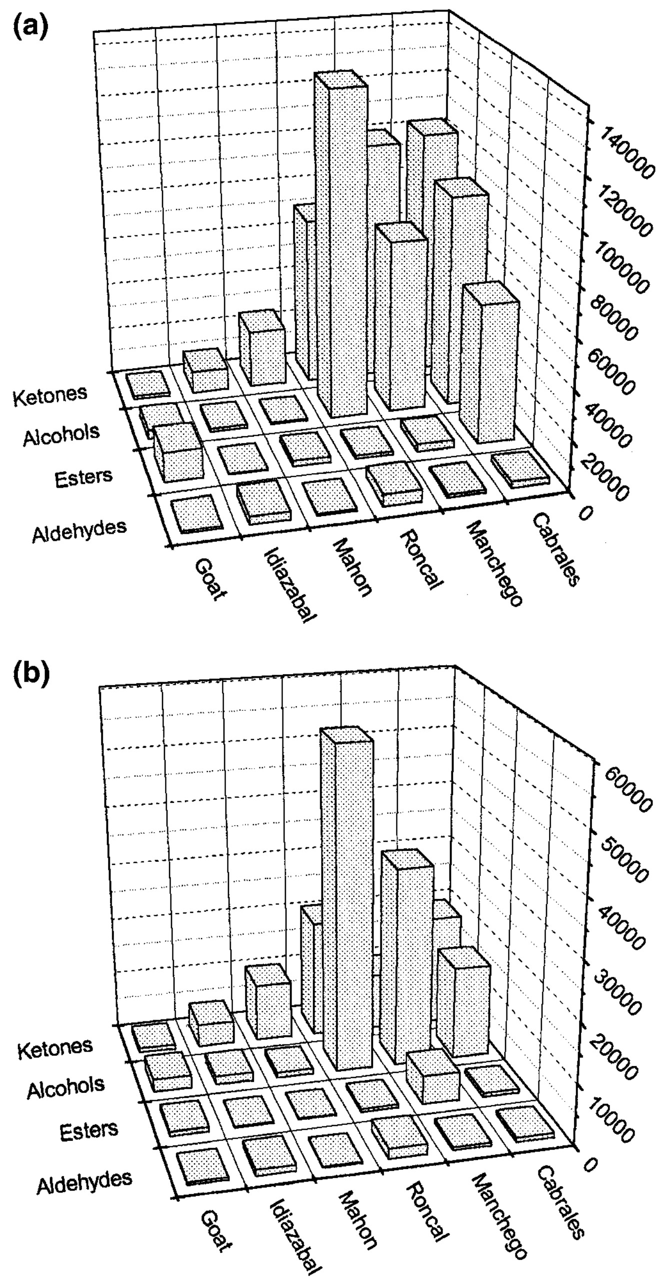

Figure 4. Total content of ketones, alcohols, aldehydes, and esters in the WSF (a) and WSF < 1000 Da (b) of goat's milk, Cabrales, Idiazábal, Mahón, Manchego, and Roncal cheeses.

The concentration of tyramine was less than or around 10 mg/100 g of DM except in Manchego cheese, which presented a value of 86.8 mg/100 g of DM.

Volatile Compounds. The volatile compounds detected varied between 55 and 83, depending on the cheese type. They belonged to known chemical classes such as ketones, alcohols, aldehydes, esters, sulfur compounds, hydrocarbons, and others. These compounds appear in most cheese varieties (*5*), but their relative proportions, which define a specific aroma for each variety (*32*), were different. **Figure 4** shows the results obtained for volatiles in both WSF (**Figure 4a**) and WSF < 1000 Da (**Figure 4b**). Compounds such as ethanol, acetone, and ethyl acetate have not been accounted for, because their level was influenced in part by the laboratory environment.

Volatiles in the WSF were characteristic for every cheese. Cabrales had the highest content of total volatiles, mainly ketones, alcohols, and esters; Roncal showed the highest alcohol content, due to a very high peak for 2-butanol; Manchego had a high quantity of ketones and alcohols, Mahon had the highest diacetyl content, and goat's milk cheese showed a very low profile.

Cabrales cheese displayed a higher quantity and variety of components, as is expected from a blue-veined cheese (*33*, *34*). 2-Ketones and 2-alkanols (especially 2-pentanone and 2-pentanol, both metabolites from molds) mainly contributed to the high content of ketones and alcohols. The esters content was higher than that of other cheeses, ethyl butyrate being predominant among the 14 quantified esters.

Roncal WSF was very rich in alcohols and ketones, as previously found in total cheese (*35*); the main components of these groups were butanone and 2-butanol, which can be formed from pyruvate, from lactose, or from citrate (*32*).

The volatile compounds of Mahón cheese have been previously studied using SDE: the main nonacid components were methyl ketones and ethyl esters (*36*, *37*); diacetyl was not reported, probably because it is not recovered by that technique. We found diacetyl as a main component in the WSF of Mahón, along with ethyl esters.

Data about the volatiles composition of Idiazábal cheese are very scarce: Larrayoz et al. (*38*) have reported ethyl esters, alcohols, methyl ketones, and phenols using SFE as a fractioning technique. The WSF studied here had moderate quantities of methyl ketones, 2-butanol, and aldehydes (2-methylbutanal and 3-methylbutanal), the latter being probably derived from Leu and Ile.

There exist a number of data about the volatiles composition of Manchego cheese as obtained by automatic dynamic headspace (*17*, *39*, *40*). The results found here for the WSF are compatible with the published results for the whole cheese: volatiles composition is variable depending on the manufacturing process; artisanal cheeses have usually a high content of alcohols (*40*).

WSF < 1000 Da fractions (**Figure 4b**) were less characteristic, because a part of the components present in WSF were lost. Alcohols were better recovered than ketones and esters. Esters markedly decreased and became the less abundant group, and, in general, differences between cheeses were less marked: Roncal had also the highest contents of alcohols, followed by Manchego and Cabrales; Roncal and Idiazábal had also the highest aldehyde contents, but distribution of esters was not proportional in both fractions. This can be due to the ultrafiltration process, where adsorption on membranes is a new source of variation. Thus, it appears that volatiles present in WSF of cheese can be considered as quite representative of the total volatiles in cheese.

In conclusion, the study of water-soluble fractions of these six cheeses reflects the main characteristics of each variety better than the whole cheese; thus, information about the composition and distribution of the low molecular weight soluble nitrogen compounds will be further studied in order to explain their contribution to some aspects of the cheese flavor. The different levels of peptides, amino acids, volatiles, and minerals can be used as an indicator of the flavor development. The data shown in this study indicate that the special and peculiar characteristics of each PDO Spanish cheese are related to the differences found in the WSF < 1000 Da, although volatile compounds were more characteristic in the WSF.

ABBREVIATIONS USED

WSF, water-soluble fraction; WSF < 1000 Da, water-soluble fraction lower than 1000 Da; TN, total nitrogen; WSN, water-soluble nitrogen; DM, dry matter; IDF, International Dairy Federation.

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