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ORIGINAL ARTICLE

Volatile compounds of Van Herby cheeses produced with raw and pasteurized milks from different species

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Abstract Levels of volatile compounds in Van herby cheeses manufactured from raw and pasteurized; 100 % ewes', 50 % ewes'+50 % cows' and mixture of 50 % ewes'+25 % cows'+ 25 % goats' milks were investigated over 180 days of ripening at 4 °C. The volatile compounds levels of herby cheese samples increased throughout the 180 days storage period. Samples produced from pasteurized milk showed lower volatile contents than their counterparts produced from raw milk. The volatile compounds profile of herby cheese samples detected by headspace solid-phase microextraction (HS-SPME) consisted of 8 esters, 5 ketones, 5 aldehydes, 9 acids, 6 alcohols and 14 hydrocarbons and terpenes. Acetic acid was the most abundant volatile compound in HS-SPME of ripened cheeses, followed by hexanoic, octanoic and butanoic acids.

Keywords Herby cheese · Volatile compounds · HS-SPME · Cheese

Introduction

Van herby cheese is one of the most common and highly appreciated dairy products in rural regions of Turkey (Eastern Anatolia), and it has an increasing popularity all over the country. In Turkey, herby cheese is generally produced under unhygenic and primitive conditions in rural region and it is usually marketed through informal route such as open markets and street vending. Van herby cheese is a semi hard cheese type which is produced from raw ewe milk. If ewe milk is not available, a mixture of cow and goat milk can be used. The most common herbs used in herby cheese production belong to *Liliaceae* (*Allium* sp.), *Apiaceae* (*Ferula sp.*, *Anthriscus nemorosa*) *and Lamiaceae* (*Thymus migricus*) families. The herbs give the cheese its characteristic appearance and aroma, but also extend the shelf life of the cheese. Herby cheese is ripened in brine or in earthenware or plastic containers using dry-salting. Dry-salted cheeses are ripened under soil during ripening (Coskun 1998; Tarakci et al. 2004; Coskun and Ozturk 2000).

During cheese ripening, numerous chemical and biochemical reactions occur. The development of the flavor and texture of cheese is achieved by the interplay of three biochemical events (proteolysis, lipolysis and glycolysis) along with numerous concomitant secondary transformations during cheese ripening (Fox et al. 1993; Law 1984).

Esters, ketones, alcohols, lactones and sulfur compounds are the main aroma-impact compounds of cheese. In cheese manufacturing, aroma is influenced by different factors; among them, milk origin and treatment (raw and pasteurized milk) are important parameters (Berard et al. 2007).

The chemical and microbiological properties of herby cheese were investigated by several authors (Coskun and Tuncturk 2000; Coskun 1998; Tarakci et al. 2004), however, to the best of authors' knowledge there are no reports related to the effects of different milk species (ewes', cows' and goats') and treatments (raw and pasteurized) on volatile composition of herby cheese during ripening. Therefore, there is a need to thoroughly investigate the properties of herby cheese to ensure product safety, high quality standards, and consequently national and international recognition in the marketing of herby cheese.

The purpose of the present study was to examine changes in volatile compounds during ripening of Van herby cheese made from ewes', ewes'and cows', and mixture of ewe-cow-goat milks.

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Materials and methods

Experimental material The ewe, cow and goat milks used in this study were supplied from local dairy plant in Van, Turkey. Cheese production was carried out in Pilot Dairy Plant of Food Engineering Department of Yuzuncu Yil University. Commercial animal rennet (Mayasan Company, Istanbul, Turkey) was used as coagulant. Freeze-dried starter cultures were obtained from Peyma-Chr. Hansen (Istanbul, Turkey). Mesophilic starter was a blend of *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* in equal proportions. Herbs [*Allium* sp. (local name sirmo), *Ferula* sp. (heliz).,and *Chaerophyllum macropodum* Boiss.,(mendo)] in pickled form were purchased from a local market in Van. All chemicals used in this study were supplied from Sigma-Aldrich Chemicals (Milwaukee, WI, USA).

Cheese making and sampling Two different manufacturing methods were applied for producing herby cheeses. 100 % ewe (A), 50 % ewe+50 % cow (B), and 50 % ewe+ 25 % cow+ 25 % goat milk (C) produced with raw (1 subscripts) and pasteurized (2 subscripts) milks were separately used to produce experimental herby cheeses. In the first method (1), milk and herbs were not pasteurized and the starter culture was not used. Milks was filtered and warmed up to 32 °C. Then, it was coagulated by using calf rennet. After coagulation, the curd was cut into small cubes and herbs was added at ratio of 2 % of cheese-milk weight and mixed well. Thereafter, the curds were drained and pressed for 3 h. When pressing was finished, the curd was cut into the blocks of 7×7×2 cm. Cheeses were brine-salted at 4 °C for 180 days in 14 % (w/v) brine concentration. In the second method (2), milk was pasteurized at 65 °C for 30 min. Then milk was cooled to 32 °C and $CaCl_2$ and starter culture (*Lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris*) were added at the ratios of 0.02 and 1.5 %, respectively. Thereafter, milk was coagulated with rennet enzyme (1/12000). After proper coagulation, the curd formed was cut into small cubes (about 1 cm^3). The pasteurized herbs (at 65 °C for 30 min) was added into the curd and mixed well. Then the curd was pressed for 3 h to remove whey. When pressing was finished, the curd was cut into the blocks of 7×7×2 cm. Cheeses were brine-salted at 4 °C for 180 days in 14 % (w/v) brine concentration. FFA and volatile composition of samples were analyzed after 2, 90 and 180 days of storage at 4 °C. The experiment was repeated twice and the analysis was triplicated.

HS-SPME-GC analysis

A 2-cm (50/30 μm divinylbenzene/carboxen/polydimethylsiloxane) SPME fiber (Supelco Co., Bellefonte, PA, USA) was used for the extraction of volatile compounds from herby cheese. The fiber was conditioned in a GC injection port at 270 °C for 1 h before use. A layer of 0.3 cm was removed from the surface of cheese and 2 g of grated sample was taken and replaced in a 30-mL screw-top headspace glass vial (I-Chem, Rockwood, TS, USA) with secure seal and 2 μg of 5-methyl 2-hexanone was added as Internal Standard (IS). After 5 min equilibration at 40 °C with constant stirring using a digital hot plate (Heidolph Instruments Gmbh & Co. KG, Schwabach, Germany), the SPME fiber was exposed for 30 min to the headspace for volatile extraction at the same temperature. After the extraction, the volatiles were desorbed in the injection port of an Agilent 6890 series GC (Agilent Technologies, Palo Alto, CA). The injector temperature was set at 250 °C and splitless injection mode used for 5 min. The samples were analyzed on a HP-Innowax column (30 m, 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). Helium was used as the carrier gas at a constant flow rate of 2 $mL.min^{-1}$. The oven temperature was programmed at 35 °C for a hold of 5 min and increased to 75 °C at a rate of 8 $°C.min^{-1}$, then increased to 220 °C at a rate of 40 $°C.min^{-1}$ and hold at the final temperature for 20 min. The Flame Ionization Detector (FID) temperature was 270 °C. All of the samples were analyzed in triplicate, and the same fiber was used for the entire experiment (Javidipour and Qian 2008). IS peak area ratio was used as an arbitrary unit for calculating the quantities of each volatile compound. Identification of volatile compounds was performed with GC-mass spectrometry (GC-MS) and retention time of authentic compounds.

GC-MS analysis

The mass spectra of herby cheese samples were obtained using an Agilent 6890 GC equipped with a 5973 mass selective detector (Agilent Technologies, Palo Alto, CA). Separation was performed on HP-Innowax column (30 m, 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). The same oven program as GC-FID analysis was performed. Helium was used as the carrier gas. The injector temperature was 250 °C. The instrument control and data analysis were performed using enhanced Chemstation software (Agilent Technologies, Inc., Santa Clara, CA, USA). Peak identification of volatiles was based on comparison of the MS spectra with the Wiley7n and Flavor2 libraries and with the spectra of injected standards as well as on the retention times of the standards (Javidipour and Qian 2008).

Statistical analysis

Statistical analysis was performed using the SAS software program (SAS/STAT 2006). Data was analyzed using two-way analysis of variance (ANOVA) and Tukey' multiple range tests.

Results and discussion

Volatiles

Total ion chromatograms (TIC) peak areas in arbitrary units for volatile compounds recovered from herby cheeses produced from different milk sources stored at 4 °C during 180 days ripening period are given in Tables 1, 2, 3, 4, 5 and 6. 49 volatile compounds including 8 esters, 5 ketones, 6 aldehydes, 9 acids, 6 alcohols and 14 hydrocarbons and terpenes were recovered from headspace of herby cheese samples by SPME. Even though TIC peak areas do not represent the true concentrations of the respective volatile compounds, they do indicate in a general way the effect of milk type, storage and duration on the formation of volatile compounds. The ratio of Peak Area/Internal Standard Peak Area was used as arbitrary units to calculate quantities for each compound (Munoz et al. 2003).

Esters Ethyl acetate, formic acid ethyl ester, ethyl butanoate, propyl butanoate, isoamyl acetate, ethyl octanoate, hexanoic acid ethyl ester and butanoic acid hexyl ester were the esters recognized in HS-SPME of herby cheese (Table 1). Butanoic acid hexyl ester was the most abundant ester found in fresh samples, hexanoic acid ethyl ester was the dominant ester in 180 days old cheeses followed by ethyl butanoate and butanoic acid hexyl ester. The relative peak area of all the ester compounds showed increasing trend during storage period. Engels et al. (1997) noted that the high concentrations of ethyl butanoate were found in fruity-tasting cheeses; Gruyere, Parmesan and Proosdij. The levels of Ethyl butanoate and butanoic acid hexyl ester in cheeses made from pasteurized milk (A2-B2-C2) were lower than those of cheeses made from raw milk (A1-B1-C1) ($P<0.05$) which were in agreement with the results reported by Rehman et al. (2000) for Cheddar cheeses, Fernandez-Garcia et al. (2002)

Table 1 Ester concentration of cheeses made from raw (A1, B1, C1) and pasteurized milk (A2, B2, C2) during ripening

Esters	Days	Cheeses					
		A_1	A_2	B_1	B_2	C_1	C_2
Formic acid ethyl ester	2	0.28 ± 0.09^{abB}	0.20 ± 0.04^{bA}	0.41 ± 0.03^{aC}	0.17 ± 0.04^{bB}	0.30 ± 0.01^{abB}	0.17 ± 0.04^{bB}
	90	0.65 ± 0.01^{aA}	0.39 ± 0.11^{bcA}	0.63 ± 0.01^{aB}	0.34 ± 0.03^{cA}	0.61 ± 0.09^{abAB}	0.31 ± 0.02^{cAB}
	180	0.86 ± 0.09^{aA}	0.51 ± 0.14^{abcA}	0.85 ± 0.03^{aA}	0.40 ± 0.03^{bcA}	0.76 ± 0.16^{abA}	0.36 ± 0.05^{cA}
Ethyl acetate	2	0.09 ± 0.03^{aA}	0.03 ± 0.00^{aB}	0.09 ± 0.03^{aA}	0.04 ± 0.01^{aA}	0.09 ± 0.00^{aB}	0.03 ± 0.01^{aA}
	90	0.18 ± 0.04^{aA}	0.06 ± 0.01^{bA}	0.13 ± 0.02^{abA}	0.06 ± 0.01^{bA}	0.17 ± 0.02^{aAB}	0.05 ± 0.01^{bA}
	180	0.22 ± 0.09^{aA}	0.07 ± 0.00^{aA}	0.19 ± 0.04^{aA}	0.08 ± 0.03^{aA}	0.21 ± 0.03^{aA}	0.07 ± 0.01^{aA}
Ethylbutanoate	2	0.36 ± 0.13^{aB}	0.15 ± 0.01^{aB}	0.35 ± 0.04^{aC}	0.18 ± 0.02^{aB}	0.30 ± 0.01^{aC}	0.17 ± 0.03^{aB}
	90	0.93 ± 0.07^{aAB}	0.35 ± 0.09^{bAB}	0.79 ± 0.10^{aB}	0.39 ± 0.03^{bA}	0.78 ± 0.15^{aB}	0.38 ± 0.02^{bA}
	180	1.23 ± 0.19^{aA}	0.50 ± 0.07^{bA}	1.09 ± 0.06^{aA}	0.47 ± 0.05^{bA}	1.01 ± 0.05^{aA}	0.47 ± 0.02^{bA}
Propylbutanoate	2	0.26 ± 0.01^{aB}	0.09 ± 0.00^{aB}	0.19 ± 0.04^{aA}	0.09 ± 0.01^{aB}	0.23 ± 0.11^{aA}	0.11 ± 0.01^{aA}
	90	0.60 ± 0.16^{aAB}	0.26 ± 0.04^{aA}	0.46 ± 0.15^{aA}	0.20 ± 0.03^{aA}	0.49 ± 0.16^{aA}	0.19 ± 0.04^{aA}
	180	0.72 ± 0.06^{aA}	0.33 ± 0.00^{abA}	0.58 ± 0.23^{abA}	0.24 ± 0.03^{bA}	0.67 ± 0.15^{abA}	0.24 ± 0.05^{bA}
Isoamylacetate	2	0.22 ± 0.02^{abB}	0.09 ± 0.02^{bcB}	0.23 ± 0.03^{aA}	0.12 ± 0.04^{abcA}	0.23 ± 0.02^{aA}	0.08 ± 0.05^{cA}
	90	0.46 ± 0.07^{aA}	0.09 ± 0.04^{bB}	0.13 ± 0.08^{bA}	0.05 ± 0.03^{bA}	0.15 ± 0.09^{bA}	0.06 ± 0.03^{bA}
	180	0.36 ± 0.06^{abAB}	0.25 ± 0.02^{aA}	0.28 ± 0.01^{aA}	0.23 ± 0.10^{aA}	0.35 ± 0.05^{aA}	0.22 ± 0.04^{aA}
Hexanoic acid ethyl ester	2	0.44 ± 0.01^{abB}	0.24 ± 0.01^{bB}	0.53 ± 0.14^{abC}	0.30 ± 0.11^{abA}	0.64 ± 0.13^{aB}	0.32 ± 0.11^{abA}
	90	2.54 ± 0.15^{aA}	0.58 ± 0.10^{cAB}	1.67 ± 0.03^{bB}	0.46 ± 0.07^{cA}	2.02 ± 0.18^{bA}	0.54 ± 0.17^{cA}
	180	2.19 ± 0.19^{aA}	0.79 ± 0.11^{bA}	2.39 ± 0.14^{aA}	0.61 ± 0.10^{bA}	2.18 ± 0.32^{aA}	0.60 ± 0.11^{bA}
Ethyl octanoate	2	0.20 ± 0.06^{bcA}	0.13 ± 0.02^{cB}	0.30 ± 0.02^{abA}	0.06 ± 0.04^{c}	0.36 ± 0.03^{aA}	0.07 ± 0.04^{cA}
	90	0.35 ± 0.13^{aA}	0.19 ± 0.03^{aAB}	0.16 ± 0.11^{aA}	0.08 ± 0.03^{a}	0.22 ± 0.11^{aA}	0.19 ± 0.00^{aA}
	180	0.42 ± 0.04^{aA}	0.25 ± 0.00^{aA}	0.35 ± 0.11^{aA}	0.22 ± 0.08^{a}	0.39 ± 0.12^{aA}	0.23 ± 0.07^{aA}
Butanoic acid hexyl ester	2	0.78 ± 0.00^{aA}	0.39 ± 0.06^{bA}	0.67 ± 0.00^{aA}	0.36 ± 0.02^{bA}	0.68 ± 0.00^{aA}	0.30 ± 0.06^{bA}
	90	1.16 ± 0.26^{aA}	0.47 ± 0.02^{bA}	0.91 ± 0.15^{abA}	0.41 ± 0.01^{bA}	1.05 ± 0.16^{aA}	0.41 ± 0.06^{bA}
	180	1.15 ± 0.31^{aA}	0.47 ± 0.11^{aA}	1.10 ± 0.32^{aA}	0.47 ± 0.06^{aA}	1.11 ± 0.17^{aA}	0.47 ± 0.04^{aA}

Data are expressed as relative peak chromatographic area. Mean values followed by different lowercase letters within a row indicate significant differences between cheese samples ($p<0.05$)

Mean values followed by different uppercase letters within a column for same compound indicate significant differences between ripening periods ($p<0.05$)

(A): 100 % ewe milk, (B): 50 % ewe milk + % 50 cow milk, (C): 50 % ewe milk + 25 % cow milk + 25 % goat milk

Table 2 Organic acid concentration of cheeses made from raw (A1, B1, C1) and pasteurized milk (A2, B2, C2) during ripening

Acids	Days	Cheeses					
		A_1	A_2	B_1	B_2	C_1	C_2
Acetic acid	2	7.31 ± 0.36^{aB}	2.17 ± 0.06^{bB}	7.54 ± 0.28^{aA}	1.53 ± 0.23^{bB}	7.00 ± 0.64^{aA}	1.53 ± 0.23^{bA}
	90	11.86 ± 3.29^{aAB}	3.68 ± 0.17^{bA}	7.80 ± 2.45^{abA}	3.51 ± 0.02^{bA}	10.37 ± 2.06^{abA}	3.11 ± 0.86^{bA}
	180	21.89 ± 4.02^{aA}	3.96 ± 0.57^{cA}	12.97 ± 0.49^{bA}	3.06 ± 0.37^{cA}	12.15 ± 1.53^{bA}	3.26 ± 0.44^{cA}
Propionic acid	2	0.30 ± 0.12^{aB}	0.22 ± 0.06^{aA}	0.21 ± 0.09^{Aa}	0.12 ± 0.02^{aB}	0.20 ± 0.07^{aB}	0.08 ± 0.04^{aB}
	90	0.73 ± 0.19^{aAB}	0.29 ± 0.04^{aA}	0.53 ± 0.20^{aA}	0.23 ± 0.03^{aAB}	0.54 ± 0.18^{aA}	0.22 ± 0.01^{aA}
	180	1.09 ± 0.10^{aA}	0.57 ± 0.21^{abA}	0.79 ± 0.21^{abA}	0.34 ± 0.04^{bA}	0.65 ± 0.13^{abA}	0.25 ± 0.00^{bA}
Butanoic acid	2	2.30 ± 0.26^{aB}	0.78 ± 0.11^{bB}	2.40 ± 0.24^{aB}	0.74 ± 0.26^{bA}	2.20 ± 0.07^{aB}	0.74 ± 0.15^{bA}
	90	4.37 ± 0.07^{aB}	1.47 ± 0.27^{bB}	3.44 ± 0.68^{aB}	1.52 ± 0.33^{bA}	3.73 ± 0.73^{aB}	1.11 ± 0.43^{bA}
	180	10.64 ± 2.30^{aA}	2.80 ± 0.22^{bA}	7.93 ± 0.06^{aA}	2.44 ± 0.99^{bA}	7.71 ± 0.58^{aA}	1.85 ± 0.69^{bA}
Hexanoic acid	2	5.10 ± 0.20^{aB}	1.60 ± 0.13^{cB}	3.95 ± 0.08^{bB}	1.46 ± 0.07^{cC}	4.26 ± 0.46^{abB}	1.33 ± 0.11^{cB}
	90	7.07 ± 0.17^{aB}	2.49 ± 0.12^{bAB}	5.26 ± 1.04^{aB}	2.67 ± 0.01^{bB}	5.39 ± 0.57^{aB}	1.86 ± 0.11^{bB}
	180	16.54 ± 1.76^{aA}	3.59 ± 0.58^{bA}	15.13 ± 1.27^{aA}	3.93 ± 0.25^{bA}	14.92 ± 2.82^{aA}	2.86 ± 0.19^{bA}
Octanoic acid	2	1.53 ± 0.20^{aC}	0.51 ± 0.05^{bB}	1.38 ± 0.04^{aC}	0.49 ± 0.07^{bB}	1.28 ± 0.11^{aC}	0.50 ± 0.06^{bB}
	90	5.94 ± 0.07^{aB}	2.92 ± 0.03^{cdA}	4.10 ± 0.64^{bcB}	2.15 ± 0.14^{dA}	4.30 ± 0.37^{bB}	1.95 ± 0.38^{dA}
	180	14.10 ± 1.51^{aA}	3.04 ± 0.42^{cA}	6.81 ± 0.70^{bA}	2.38 ± 0.21^{cA}	8.28 ± 0.60^{bA}	2.20 ± 0.28^{cA}
Nonanoic acid	2	0.06 ± 0.00^{aA}	0.03 ± 0.00^{aA}	0.05 ± 0.03^{aA}	0.03 ± 0.00^{aA}	0.06 ± 0.02^{aA}	0.03 ± 0.01^{aA}
	90	0.15 ± 0.05^{aA}	0.05 ± 0.00^{aA}	0.08 ± 0.03^{aA}	0.05 ± 0.01^{aA}	0.09 ± 0.03^{aA}	0.05 ± 0.02^{aA}
	180	0.13 ± 0.02^{A}	0.06 ± 0.01^{bA}	0.12 ± 0.00^{aA}	0.06 ± 0.00^{bA}	0.13 ± 0.00^{aA}	0.06 ± 0.01^{bA}
Decanoic acid	2	0.35 ± 0.08^{aB}	0.12 ± 0.01^{bC}	0.33 ± 0.07^{abB}	0.15 ± 0.02^{abC}	0.36 ± 0.08^{aB}	0.14 ± 0.01^{abC}
	90	5.95 ± 0.21^{aA}	2.81 ± 0.08^{bcA}	4.01 ± 1.14^{abA}	2.05 ± 0.05^{bcA}	3.54 ± 0.57^{bAB}	1.42 ± 0.10^{cB}
	180	4.85 ± 1.02^{aA}	1.48 ± 0.35^{bB}	3.53 ± 0.78^{abAB}	1.69 ± 0.04^{bB}	4.32 ± 0.18^{aA}	1.78 ± 0.07^{bA}
Benzoic acid	2	0 ± 0^{A}	0 ± 0^{A}	0 ± 0^{A}	0 ± 0^{A}	0 ± 0^{A}	0 ± 0^{B}
	90	0.03 ± 0.05^{aA}	0.02 ± 0.02^{aA}	0.03 ± 0.05^{aA}	0.03 ± 0.02^{aA}	0.04 ± 0.03^{aA}	0.03 ± 0.01^{aAB}
	180	0.04 ± 0.05^{aA}	0.03 ± 0.04^{aA}	0.04 ± 0.05^{aA}	0.03 ± 0.01^{aA}	0.06 ± 0.03^{aA}	0.04 ± 0.01^{aA}
Dodecanoic acid	2	0 ± 0^{C}	0 ± 0^{C}	0 ± 0^{C}	0 ± 0^{B}	0 ± 0^{B}	0 ± 0^{C}
	90	0.44 ± 0.10^{aB}	0.09 ± 0.02^{cB}	0.33 ± 0.06^{abB}	0.09 ± 0.02^{cAB}	0.45 ± 0.03^{aAB}	0.16 ± 0.03^{bcB}
	180	0.71 ± 0.05^{abA}	0.25 ± 0.01^{bA}	0.73 ± 0.11^{abA}	0.28 ± 0.10^{abA}	0.87 ± 0.34^{aA}	0.34 ± 0.01^{abA}

Data are expressed as relative peak chromatographic area. Mean values followed by different lowercase letters within a row indicate significant differences between cheese samples ($p<0.05$)

Mean values followed by different uppercase letters within a column for same compound indicate significant differences between ripening periods ($p<0.05$)

(A): 100 % ewe milk, (B): 50 % ewe milk + % 50 cow milk, (C): 50 % ewe milk + 25 % cow milk + 25 % goat milk

for Manchego cheeses and Berard et al. (2007) for Fontina Valle d'Aosta cheeses.

Esters which are common volatile compounds of cheeses generally have fruity odors and may also influence flavor of many varieties of cheeses such as soft cheeses, Italian and Swiss-type cheeses (Liu et al. 2004). Ethyl butanoate, ethyl pentanoate, ethyl hexanoate, and isoamyl butanoate were the aroma-impact esters analyzed in the headspace of Cheddar, hard grating and mould-ripened blue cheeses (Frank et al. 2004). Esters are formed by the condensation of an acid and an alcohol. In cheese, this reaction may be spontaneous, or may be mediated by microbial esterases (Beuvier and Buchin 2004).

Organic acids Acetic, propionic, butanoic, pentanoic, hexanoic, octanoic, nonanoic, decanoic, benzoic and dodecanoic acids recognized in headspace-SPME of herby cheese samples (Table 2).

The significant ($P<0.05$) increase in relative areas of organic acids during ripening period, and lower levels of these compounds in pasteurized batches than those produced from raw milks also observed in HS-SPME profiles of cheese samples. Acetic acid was the main acid recognized in fresh and ripened samples followed by hexanoic, octanoic, butanoic and decanoic acids. Barron et al. (2005) noted that acids (butanoic, acetic, hexanoic etc.) were the most abundant volatile compounds in the headspace of Idiazabal (90 %), Roncal (81 %) and Zamorano (78 %) cheeses. Acetic, isovaleric (3-methyl butanoic acid), butanoic, hexanoic, and pentanoic acid were acids found in the SPME profile of Cheddar, hard grating and mould-ripened blue cheeses. Butanoic acid with a

Table 3 Aldehydes concentration of cheeses made from raw (A1, B1, C1) and pasteurized milk (A2, B2, C2) during ripening

Aldehydes	Days	Cheeses					
		A_1	A_2	B_1	B_2	C_1	C_2
Acetaldehyde	2	$0.30±0.10^{aA}$	$0.11±0.03^{aA}$	$0.35±0.07^{aA}$	$0.18±0.02^{aA}$	$0.28±0.07^{aA}$	$0.15±0.02^{abB}$
	90	$0.54±0.14^{aA}$	$0.22±0.06^{aA}$	$0.54±0.18^{aA}$	$0.30±0.09^{aA}$	$0.48±0.06^{aA}$	$0.22±0.01^{aA}$
	180	$0.70±0.12^{aA}$	$0.29±0.08^{aA}$	$0.73±0.22^{aA}$	$0.35±0.10^{aA}$	$0.60±0.11^{aA}$	$0.26±0.01^{aA}$
Pentanal	2	$0.28±0.04^{aA}$	$0.19±0.04^{aA}$	$0.24±0.06^{aA}$	$0.15±0.02^{aA}$	$0.27±0.03^{aA}$	$0.15±0.06^{aA}$
	90	$0.49±0.15^{aA}$	$0.33±0.08^{aA}$	$0.34±0.01^{aA}$	$0.24±0.10^{aA}$	$0.42±0.07^{aA}$	$0.24±0.02^{aA}$
	180	$0.48±0.15^{aA}$	$0.28±0.15^{aA}$	$0.37±0.13^{aA}$	$0.19±0.02^{aA}$	$0.42±0.07^{aA}$	$0.22±0.01^{aA}$
Hexenal	2	$0.22±0.12^{aA}$	$0.10±0.01^{aA}$	$0.22±0.06^{aA}$	$0.11±0.00^{aA}$	$0.19±0.06^{aA}$	$0.10±0.02^{aA}$
	90	$0.36±0.10^{aA}$	$0.15±0.00^{aA}$	$0.24±0.08^{aA}$	$0.17±0.01^{aA}$	$0.30±0.01^{aA}$	$0.16±0.03^{aA}$
	180	$0.38±0.04^{aA}$	$0.17±0.05^{bcA}$	$0.29±0.05^{abcA}$	$0.17±0.03^{cA}$	$0.33±0.06^{abA}$	$0.16±0.02^{cA}$
Hexanal	2	$0.12±0.00^{aB}$	$0.07±0.00^{aA}$	$0.13±0.04^{aA}$	$0.07±0.01^{aB}$	$0.11±0.02^{aA}$	$0.08±0.00^{aB}$
	90	$0.20±0.02^{aAB}$	$0.11±0.02^{aA}$	$0.19±0.04^{aA}$	$0.11±0.01^{aA}$	$0.19±0.00^{aA}$	$0.12±0.00^{aAB}$
	180	$0.24±0.02^{aA}$	$0.14±0.04^{abA}$	$0.22±0.00^{abA}$	$0.13±0.01^{bA}$	$0.22±0.04^{abA}$	$0.13±0.02^{bA}$
Tridecanal	2	$0.20±0.04^{bB}$	$0.10±0.02^{bB}$	$0.16±0.06^{bB}$	$0.15±0.07^{bB}$	$0.38±0.01^{aB}$	$0.17±0.03^{bB}$
	90	$3.39±0.20^{aA}$	$1.19±0.16^{bA}$	$3.45±0.83^{aA}$	$1.31±0.05^{bA}$	$2.89±0.40^{aA}$	$1.15±0.05^{bA}$
	180	$3.64±0.30^{aA}$	$1.53±0.21^{cdA}$	$2.99±0.34^{abA}$	$1.04±0.26^{dA}$	$2.42±0.25^{bcA}$	$0.91±0.33^{dAB}$

Data are expressed as relative peak chromatographic area. Mean values followed by different lowercase letters within a row indicate significant differences between cheese samples ($p<0.05$)

Mean values followed by different uppercase letters within a column for same compound indicate significant differences between ripening periods ($p<0.05$)

(A): 100 % ewe milk, (B): 50 % ewe milk + % 50 cow milk, (C): 50 % ewe milk + 25 % cow milk + 25 % goat milk

characteristic cheesy sharp aroma and hexanoic acid with a very mild to sharp goat-like smell are generally major aroma compounds of different cheeses (Frank et al. 2004). Fatty acids are released on the lipolysis of the fat, however, acetic, propionic and butanoic acids may also be produced by the fermentation of lactose and lactic acid.

Aldehydes Acetaldehyde, pentanal, hexenal, hexanal and tridecanal were the aldehydes detected in experimental herby cheese samples. Tridecanal showed higher final concentrations than the other aldehydes (Table 3). Aldehydes do not accumulate to high concentrations in cheese because due to the enzymatic activities of microorganisms, aldehydes are oxidized to acids or reduced to n-alkanols (Munoz et al. 2003; Beuvier and Buchin 2004). Methyl butanal and 2-nonenal were aldehydes detected in SPME profile of Cheddar cheese (Frank et al. 2004). Hexanal and 3-methylbutanal were the most abundant compounds detected in Fontina cheese by applying dynamic headspace (Berard et al. 2007). Branched and linear aldehydes are probably derived by the microbial degradation of amino acids (transamination followed by decarboxylation) or via Strecker degradation, while linear aldehydes are additionally formed from lipid oxidation (Bintsis and Robinson 2004).

Alcohols Ethanol, isoamyl alcohol, 2-heptanol, 2-ethyl-1hexanol, 2-nonanol, and 3-phenoxy-1-propanol were the alcohols detected in herby cheese samples. Isoamylalcohol was the major alcohol detected in herby cheese produced with different milk sources (Table 4). 2-Ethyl 1-hexanol was the most abundant alcohol detected in aged cheeses, significantly ($P<0.05$) formed during ripening. Samples produced from raw milks showed higher alcohol development during ripening than those produced from pasterurized milk. Other authors (Ortigosa et al. 2001; Rehman et al. 2000; Rodriguez-Alonso et al. 2009) have also reported that raw milk cheeses contained greater amounts of alcohols than pasteurized milk cheeses. Hayaloglu et al. (2008) reported that 32 alcohols were identified in Küflü cheeses and the alcohols present at the greatest concentrations were 3-methyl butanol, 2-heptanol and 2-nonanol. 1-Butanol, 3-methyl-butan-1-ol and phenethylalcohol were detected by Rehman et al. (2000) in Cheddar cheese made from raw and pasteurized milk. Berard et al. (2007) reported that 2-butanol was the most abundant compound detected in dynamic headspace of Fontina cheese, followed by 3-methyl-1-butanol, 1-propanol and ethanol. Alcohols may be rapidly produce form aldehydes under strong reducing condition present in cheese, or from other metabolic pathways, such as lactose metabolism and amino acids catabolism. Secondary alcohols are formed by

Table 4 Alcohol concentration of cheeses made from raw (A1, B1, C1) and pasteurized milk (A2, B2, C2) during ripening

Alcohols	Days	Cheeses					
		A_1	A_2	B_1	B_2	C_1	C_2
Ethanol	2	$0.09±0.01^{abcA}$	$0.03±0.00^{dB}$	$0.10±0.02^{abB}$	$0.04±0.01^{cdB}$	$0.12±0.02^{aB}$	$0.05±0.02^{bcdA}$
	90	$0.20±0.02^{aA}$	$0.06±0.01^{bAB}$	$0.19±0.02^{aAB}$	$0.07±0.01^{bAB}$	$0.22±0.01^{aA}$	$0.07±0.02^{bA}$
	180	$0.24±0.08^{abA}$	$0.09±0.00^{bA}$	$0.23±0.04^{abA}$	$0.09±0.01^{bA}$	$0.28±0.01^{aA}$	$0.10±0.02^{bA}$
İsoamylalcohol	2	$0.93±0.20^{abcA}$	$0.61±0.03^{bcA}$	$1.04±0.04^{abA}$	$0.51±0.07^{cA}$	$1.18±0.17^{aA}$	$0.57±0.03^{cB}$
	90	$1.66±0.05^{aA}$	$0.70±0.03^{aA}$	$2.31±1.05^{aA}$	$1.37±0.44^{aA}$	$2.34±0.82^{aA}$	$1.23±0.26^{aA}$
	180	$1.68±0.24^{aA}$	$0.70±0.24^{aA}$	$1.61±0.01^{aA}$	$0.59±0.16^{aA}$	$0.84±0.71^{aA}$	$0.70±0.07^{aAB}$
2-heptanol	2	$0.28±0.08^{aA}$	$0.14±0.07^{aA}$	$0.21±0.00^{aA}$	$0.14±0.05^{aA}$	$0.16±0.01^{aA}$	$0.12±0.05^{aA}$
	90	$0.18±0.13^{aA}$	$0.09±0.04^{aA}$	$0.19±0.16^{aA}$	$0.13±0.05^{aA}$	$0.18±0.07^{aA}$	$0.07±0.05^{aA}$
	180	$0.24±0.09^{aA}$	$0.12±0.01^{aA}$	$0.17±0.11^{aA}$	$0.09±0.04^{aA}$	$0.14±0.03^{aA}$	$0.06±0.02^{aA}$
2-ethyl 1-hexanol	2	$0.46±0.09^{aC}$	$0.13±0.05^{cC}$	$0.31±0.00^{abcB}$	$0.14±0.03^{bcB}$	$0.35±0.08^{ab}$	$0.14±0.03^{cB}$
	90	$1.86±0.33^{aB}$	$0.99±0.11^{abcB}$	$1.83±0.30^{aB}$	$0.66±0.12^{cB}$	$1.69±0.26^{ab}$	$0.94±0.03^{bcB}$
	180	$5.28±0.45^{abA}$	$2.21±0.23^{bA}$	$7.24±1.78^{aA}$	$3.52±0.33^{bA}$	$3.82±0.63^{bA}$	$2.57±0.58^{bA}$
2-nonanol	2	$0.15±0.03^{aB}$	$0.07±0.00^{aB}$	$0.13±0.05^{aB}$	$0.06±0.02^{aB}$	$0.11±0.03^{aB}$	$0.07±0.02^{aB}$
	90	$0.70±0.18^{aA}$	$0.28±0.03^{bA}$	$0.44±0.11^{abA}$	$0.29±0.03^{bA}$	$0.47±0.04^{abA}$	$0.28±0.02^{bA}$
	180	$0.10±0.01^{aB}$	$0.06±0.01^{abB}$	$0.06±0.02^{abB}$	$0.04±0.00^{bB}$	$0.08±0.02^{abB}$	$0.09±0.01^{abB}$
3-phenoxy-1-propanol	2	$0.03±0.01^{aB}$	$0.02±0.00^{aB}$	$0.04±0.01^{aC}$	$0.02±0.00^{aB}$	$0.04±0.01^{aC}$	$0.03±0.00^{aB}$
	90	$0.29±0.12^{aAB}$	$0.13±0.04^{aAB}$	$0.32±0.07^{aB}$	$0.25±0.06^{aAB}$	$0.32±0.07^{aB}$	$0.19±0.01^{aAB}$
	180	$0.55±0.07^{aA}$	$0.45±0.17^{aA}$	$0.54±0.01^{aA}$	$0.43±0.15^{aA}$	$0.75±0.00^{a}$	$0.55±0.18^{aA}$

Data are expressed as relative peak chromatographic area. Mean values followed by different lowercase letters within a row indicate significant differences between cheese samples ($p<0.05$)

Mean values followed by different uppercase letters within a column for same compound indicate significant differences between ripening periods ($p<0.05$)

(A): 100 % ewe milk, (B): 50 % ewe milk + % 50 cow milk, (C): 50 % ewe milk + 25 % cow milk + 25 % goat milk

Table 5 Ketones concentration of cheeses made from raw (A1, B1, C1) and pasteurized milk (A2, B2, C2) during ripening

Ketones	Days	Cheeses					
		A_1	A_2	B_1	B_2	C_1	C_2
2-butanone	2	$0.16±0.02^{aA}$	$0.07±0.02^{aA}$	$0.15±0.02^{aA}$	$0.08±0.00^{aB}$	$0.14±0.04^{aA}$	$0.11±0.03^{aA}$
	90	$0.26±0.01^{abA}$	$0.10±0.00^{cA}$	$0.27±0.06^{abA}$	$0.12±0.00^{cA}$	$0.28±0.06^{aA}$	$0.14±0.00^{bcA}$
	180	$0.27±0.04^{aA}$	$0.16±0.06^{aA}$	$0.27±0.05^{aA}$	$0.13±0.01^{aA}$	$0.27±0.04^{aA}$	$0.17±0.03^{aA}$
2-pentanone	2	$0.19±0.08^{aA}$	$0.11±0.00^{aAA}$	$0.17±0.03^{aB}$	$0.09±0.01^{aA}$	$0.18±0.04^{aB}$	$0.10±0.02^{aA}$
	90	$0.28±0.04^{aA}$	$0.14±0.07^{abA}$	$0.23±0.03^{abAB}$	$0.13±0.03^{bA}$	$0.27±0.02^{abAB}$	$0.15±0.02^{abA}$
	180	$0.32±0.05^{aA}$	$0.16±0.04^{bA}$	$0.31±0.04^{aA}$	$0.16±0.03^{bA}$	$0.33±0.02^{aA}$	$0.16±0.03^{bA}$
3-methyl-2-butanone	2	$0.09±0.04^{aA}$	$0.04±0.01^{aA}$	$0.10±0.01^{aB}$	$0.06±0.00^{aB}$	$0.09±0.01^{aA}$	$0.05±0.01^{aA}$
	90	$0.20±0.07^{aA}$	$0.06±0.01^{bA}$	$0.14±0.00^{abA}$	$0.09±0.00^{abA}$	$0.16±0.01^{abA}$	$0.08±0.00^{abA}$
	180	$0.23±0.02^{aA}$	$0.07±0.00^{cA}$	$0.19±0.01^{aA}$	$0.10±0.01^{bcA}$	$0.18±0.04^{abA}$	$0.09±0.01^{cA}$
2-hexanone	2	$0.16±0.04^{aA}$	$0.09±0.00^{aB}$	$0.15±0.02^{aB}$	$0.10±0.00^{aA}$	$0.14±0.04^{aB}$	$0.10±0.00^{aB}$
	90	$0.30±0.05^{aA}$	$0.14±0.02^{cA}$	$0.24±0.03^{abcAB}$	$0.15±0.01^{bcA}$	$0.26±0.03^{abAB}$	$0.14±0.01^{cAB}$
	180	$0.34±0.03^{aA}$	$0.17±0.00^{bA}$	$0.29±0.01^{aA}$	$0.15±0.02^{bA}$	$0.30±0.02^{aA}$	$0.17±0.02^{bA}$
2-heptanone	2	$0.20±0.00^{aB}$	$0.10±0.01^{bC}$	$0.23±0.00^{aA}$	$0.13±0.01^{bA}$	$0.20±0.02^{aB}$	$0.14±0.00^{bB}$
	90	$1.06±0.40^{aAB}$	$0.52±0.05^{aB}$	$1.04±0.41^{aA}$	$0.65±0.23^{aA}$	$1.07±0.07^{aA}$	$0.62±0.21^{aAB}$
	180	$1.50±0.20^{aA}$	$0.97±0.10^{aA}$	$1.46±0.33^{aA}$	$0.97±0.36^{aA}$	$1.33±0.27^{aA}$	$0.98±0.28^{aA}$

Data are expressed as relative peak chromatographic area. Mean values followed by different lowercase letters within a row indicate significant differences between cheese samples ($p<0.05$)

Mean values followed by different uppercase letters within a column for same compound indicate significant differences between ripening periods ($p<0.05$)

(A): 100 % ewe milk, (B): 50 % ewe milk + % 50 cow milk, (C): 50 % ewe milk + 25 % cow milk + 25 % goat milk

Table 6 Hydrocarbons and terpenes concentration of cheeses made from raw (A1, B1, C1) and pasteurized milk (A2, B2, C2) during ripening

Hydrocarbons and terpenes	Days	Cheeses					
		A_1	A_2	B_1	B_2	C_1	C_2
Nonadecane	2	0.13±0.01^{abB}	0.07±0.00^{bB}	0.15±0.04^{aB}	0.08±0.01^{abA}	0.15±0.03^{aA}	0.08±0.01^{abA}
	90	0.27±0.01^{bB}	0.17±0.05^{aAB}	0.20±0.08^{aB}	0.17±0.02^{aA}	0.23±0.11^{aA}	0.11±0.03^{aA}
	180	0.60±0.11^{aA}	0.24±0.03^{bcA}	0.46±0.05^{abA}	0.11±0.05^{cA}	0.19±0.07^{cA}	0.08±0.04^{cA}
Hexadecane	2	0.08±0.01^{aA}	0.03±0.00^{bB}	0.09±0.00^{aB}	0.03±0.01^{bB}	0.10±0.01^{aA}	0.03±0.02^{bA}
	90	0.19±0.05^{aA}	0.06±0.01^{bAB}	0.11±0.02^{abAB}	0.07±0.01^{bAB}	0.11±0.05^{abA}	0.08±0.01^{abA}
	180	0.22±0.03^{aA}	0.10±0.02^{bcA}	0.17±0.02^{abA}	0.11±0.02^{bcA}	0.12±0.02^{bcA}	0.07±0.02^{cA}
Caryophyllen	2	0.15±0.03^{aA}	0.10±0.02^{aAB}	0.11±0.08^{aA}	0.10±0.01^{aB}	0.10±0.07^{aA}	0.08±0.04^{aA}
	90	0.32±0.08^{aA}	0.18±0.00^{aA}	0.33±0.11^{aA}	0.23±0.03^{aA}	0.36±0.11^{aA}	0.23±0.07^{aA}
	180	0.17±0.06^{aA}	0.06±0.04^{aB}	0.09±0.05^{aA}	0.06±0.02^{aB}	0.11±0.05^{aA}	0.07±0.03^{aA}
Heptadecane	2	0.11±0.03^{aA}	0.06±0.01^{aA}	0.09±0.04^{aA}	0.11±0.05^{aA}	0.08±0.03^{aA}	0.12±0.04^{aA}
	90	0.28±0.04^{aA}	0.10±0.04^{bA}	0.17±0.04^{abA}	0.08±0.03^{bA}	0.14±0.07^{abA}	0.09±0.04^{bA}
	180	0.15±0.03^{abAB}	0.08±0.02^{bA}	0.20±0.03^{aA}	0.10±0.00^{bA}	0.13±0.03^{abA}	0.09±0.02^{bA}
Butyllated hydroxytoluen	2	0.07±0.03^{aA}	0.07±0.02^{aA}	0.14±0.06^{aA}	0.11±0.04^{aA}	0.16±0.02^{aA}	0.12±0.03^{aA}
	90	0.02±0.03^{aA}	0.02±0.02^{aAB}	0.02±0.03^{aA}	0.02±0.02^{aA}	0.03±0.04^{aB}	0.02±0.02^{aAB}
	180	0.00±0.00^{aA}	0.00±0.00^{aB}	0.00±0.00^{aA}	0.00±0.00^{aA}	0.00±0.00^{aB}	0.00±0.00^{aB}
Phenol	2	0.20±0.03^{aB}	0.12±0.05^{aB}	0.19±0.02^{aB}	0.10±0.00^{aB}	0.17±0.05^{aB}	0.08±0.02^{aB}
	90	0.79±0.07^{aA}	0.038±0.03^{cA}	0.52±0.03^{bcA}	0.35±0.06^{cA}	0.61±0.08^{abA}	0.36±0.05^{cA}
	180	0.28±0.08^{aAB}	0.11±0.02^{bB}	0.24±0.04^{abB}	0.18±0.01^{abB}	0.24±0.04^{abB}	0.14±0.01^{abB}
p-cresol	2	0.14±0.01^{aAB}	0.07±0.02^{abA}	0.07±0.02^{abB}	0.04±0.02^{bA}	0.08±0.05^{abA}	0.04±0.01^{bA}
	90	0.10±0.03^{aB}	0.05±0.01^{aA}	0.12±0.03^{aB}	0.04±0.02^{aA}	0.10±0.03^{aA}	0.07±0.01^{aA}
	180	0.21±0.03^{aA}	0.11±0.01^{bA}	0.23±0.01^{aA}	0.07±0.02^{bA}	0.12±0.04^{bA}	0.07±0.01^{bA}
m-cresol	2	0.13±0.01^{aB}	0.04±0.00^{aC}	0.15±0.03^{aA}	0.06±0.01^{aA}	0.15±0.07^{aA}	0.06±0.01^{aA}
	90	0.29±0.03^{Aa}	0.15±0.01^{bA}	0.11±0.00^{bcA}	0.05±0.01^{cA}	0.13±0.03^{bcA}	0.07±0.01^{cA}
	180	0.18±0.03^{abB}	0.07±0.01^{bB}	0.25±0.07^{aA}	0.05±0.02^{bA}	0.15±0.02^{abA}	0.06±0.01^{bA}
Piperitenone	2	0.09±0.00^{aC}	0.03±0.00^{cC}	0.05±0.00^{bB}	0.03±0.00^{cC}	0.05±0.00^{bB}	0.03±0.00^{cC}
	90	1.38±0.13^{abcB}	0.64±0.10^{dB}	1.44±0.32^{abA}	0.78±0.03^{bcdA}	1.49±0.21^{aA}	0.71±0.00^{cdA}
	180	2.75±0.27^{aA}	1.09±0.04^{cA}	1.74±0.20^{bA}	0.44±0.07^{dB}	1.70±0.17^{bcA}	0.40±0.01^{dB}
Thymol	2	0.07±0.00^{aA}	0.04±0.01^{aB}	0.06±0.01^{aB}	0.04±0.01^{aB}	0.06±0.02^{aA}	0.05±0.01 ^{aB}
	90	0.21±0.04^{aA}	0.14±0.00^{aA}	0.20±0.03^{aA}	0.13±0.02^{aA}	0.17±0.05^{aA}	0.10±0.00^{aA}
	180	0.18±0.02^{aA}	0.08±0.02^{cB}	0.17±0.02^{aA}	0.09±0.00^{bcAB}	0.15±0.02^{abA}	0.10±0.00^{bcA}
α-pinene	2	0.05±0.01^{bB}	0.06±0.03^{aA}	0.07±0.01^{aB}	0.06±0.01^{aA}	0.08±0.01^{aB}	0.07±0.00^{aB}
	90	0.18±0.02^{aA}	0.09±0.03^{bA}	0.12±0.03^{abAB}	0.10±0.00^{bA}	0.15±0.02^{abA}	0.10±0.00^{bA}
	180	0.25±0.02^{aA}	0.13±0.04^{bA}	0.14±0.00^{bA}	0.10±0.02^{bA}	0.17±0.02^{abA}	0.12±0.02^{bA}
β-pinene	2	0.05±0.01^{aA}	0.03±0.01^{aA}	0.04±0.01^{aA}	0.03±0.00^{aA}	0.05±0.01^{aB}	0.03±0.01^{aB}
	90	0.12±0.04^{aA}	0.05±0.01^{aA}	0.07±0.02^{aA}	0.05±0.01^{aA}	0.11±0.01^{aA}	0.05±0.00^{aAB}
	180	0.14±0.02^{aA}	0.06±0.01^{bA}	0.10±0.03^{abA}	0.06±0.02^{bA}	0.11±0.01^{abA}	0.06±0.01^{bA}
Limonene	2	0.25±0.08^{aA}	0.20±0.04^{aA}	0.24±0.08^{aA}	0.12±0.02^{aA}	0.23±0.07^{aA}	0.12±0.01^{aA}
	90	0.26±0.10^{aA}	0.19±0.04^{aA}	0.24±0.08^{aA}	0.17±0.04^{aA}	0.27±0.12^{aA}	0.16±0.04^{aA}
	180	0.47±0.08^{aA}	0.23±0.05^{aA}	0.34±0.21^{a}	0.20±0.01^{aA}	0.42±0.13^{aA}	0.25±0.10^{aA}
Styrene	2	0.55±0.04^{aB}	0.21±0.01^{bC}	0.46±0.08^{aA}	0.21±0.01^{bB}	0.45±0.02^{aB}	0.22±0.04^{bB}
	90	0.86±0.00^{aA}	0.35±0.02^{cB}	0.69±0.11^{abA}	0.39±0.02^{cAB}	0.78±0.13^{abAB}	0.48±0.08^{bcA}
	180	0.72±0.12^{aAB}	0.77±0.05^{aA}	0.74±0.23^{aA}	0.75±0.18^{aA}	1.06±0.17^{aA}	0.68±0.07^{aA}

Data are expressed as relative peak chromatographic area. Mean values followed by different lowercase letters within a row indicate significant differences between cheese samples (*p<0.05*)

Mean values followed by different uppercase letters within a column for same compound indicate significant differences between ripening periods (*p<0.05*)

(A): 100 % ewe milk, (B): 50 % ewe milk + % 50 cow milk, (C): 50 % ewe milk + 25 % cow milk + 25 % goat milk

enzymatic reduction of methyl ketones which are produced from fatty acids (Molimard and Spinnler 1996). The presence of the branched-chain primary alcohols, 2-methyl-1-butanol, 2-methyl-1-propanol and 3-methyl-1-butanol indicates conversion of the aldehydes produced from catabolism of Ile, Val and Leu, respectively (Engels et al. 1997). Alcohols indirectly can be responsible for cheese flavor because of their ability to form esters with free fatty acids (Foda et al. 2008).

Ketones 2-Butanone, 2-pentanone, 3-methyl-2-butanone, 2-hexanone and 2-heptanone were the ketones extracted from headspace of herby cheese by SPME. All of the ketones (except 2-heptanone) showed lower initial and final concentrations than their counterparts produced from raw milks (Table 5). High concentrations of 2-heptanone, 2-nonanone and 2-octanone were measured in SPME profile of Blue cheese (Frank et al. 2004). 2-Pentanone and 2-nonanone were also detected by Bintsis and Robinson (2004) in Feta-type cheese. Moulds (e.g. *Penicillium roqueforti*) are responsible for the conversion of FFAs into methyl ketones in Blue-mould-type cheeses (Gehrig and Knight 1963). It has been suggested that Methyl ketones are not only originate from the β-oxidation of fatty acids by microorganisms, they can also be formed from ketoacids naturally present at low concentrations in milk fat or by oxidation of monounsaturated fatty acids. Their aromatic impact is of primary importance in blue and surface mould-ripened cheeses, but they are also likely to have an influence on the flavor of other varieties (Beuvier and Buchin 2004).

Hydrocarbons and terpenes A total of 14 hydrocarbons and terpenes were detected in headspace of Van herby cheeses; however; generally no significant differences were found among the cheeses (Table 6). This group of secondary products of lipid autoxidation does not make a major contribution to aroma, although these compounds may serve as precursors for the formation of other aromatic compounds (Munoz et al. 2003). The concentrations of piperitenone and styrene were higher than the concentrations of the other compounds in 90 and 180 days of ripening. Piperitenone is a phytochemical found in plants belonging to the Lamiaceae (Ghoulami et al. 2001). Styrene can be produced from Phe (Molimard and Spinnler 1996). All of the components were found higher in cheeses made from raw milk than in cheeses made from pasteurized milk.

Hydrocarbons and terpenes are a group of lipophilic aliphatic compounds, originated from the secondary metabolism of plants (Foda et al. 2008; Abilleira et al. 2010). Negi (2012) reported that herbs are contained many phytochemicals (steroids, terpenoids, flavonoids, phenolics etc.). Therefore, hydrocarbons and terpenes can be rapidly transferred into milk fat by forages or the herbs added to cheese.

Conclusion

This work provides the first characterization of the HS-SPME profile of Van herby cheese produced with raw and pasteurized milks from different species. Generally, the concentrations of volatiles in cheese samples increased during storage. Cheeses produced from pasteurized milk showed lower volatile compounds development during ripening than their counterparts produced from raw milk. Cheeses produced from raw ewe milk showed the highest volatile levels among the experimental cheeses. Some volatiles formed during biochemical reactions, some others came directly from added herbs. Acetic acid, followed by hexanoic, octanoic and butanoic acids were the major volatile compounds in HS-SPME of ripened cheeses.

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