

VOLATILE COMPOUNDS IDENTIFIED IN TRADITIONAL CROATIAN COW'S AND GOAT'S CHEESES MATURED IN LAMBSKIN SACK DETERMINED BY GC-MS ANALYSIS

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Abstract. Cheeses ripened in animal skin sacks belong to traditional cheeses that are strongly connected to the culture and history of the country from which they originate. Their main specificity is anaerobic ripening in an animal skin sack. The aim of this study was to determine the volatile compounds of Croatian cheeses in a sack of lamb skin produced from either raw cow's or goat's milk and relate them to the aroma of these traditional cheeses. Volatile compounds were extracted by headspace solid-phase microextraction (HS-SPME) and ultrasonic solvent extraction (USE) and analysed by gas chromatography-mass spectrometry (GC-MS). A total of 32 volatiles were identified in the cheese samples, including 12 carboxylic acids, 8 esters, 6 alcohols, 3 ketones, 2 hydrocarbons and 1 aldehyde. In the samples obtained by HS-SPME, the fatty acids and alcohols were the most abundant, while in the samples obtained by USE, the fatty acids were the most abundant.

Keywords: headspace solid-phase microextraction, ultrasonic solvent extraction, traditional cheese.

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Introduction

Traditional products have specific characteristics that distinguish them from other similar products in the same category, due to the use of traditional ingredients, traditional production methods and/or processing methods. Recently, there has been an increasing interest in the consumption of traditional cheeses, mainly because no additives are used in their production and they have a positive impact on human health [1]. Cheeses matured in animal skin sacks belong to traditional cheeses that are closely associated with the culture and history of the country of their origin. Several countries produce different types of cheese matured in animal skin sacks. They differ depending on the production process and the type of milk used. They also have different local names, such as “Sir iz mišine” (Croatia), “Sir iz mijeha” (Bosnia and Herzegovina and Montenegro), “Tulum” (Turkey) and “Chekoua” (Algeria). The most important characteristic of these cheeses is anaerobic ripening in a sack made from the skin of the whole lamb or goat [2].

The production of cheese in a sack has a long tradition in the Croatian region of Dalmatia. Historically, it dates back to the time of the ancient Illyrians and Thracians, who used lambskins to store and transport milk from the

Mount Dinara to the valley. The cheese was probably created by accident when the rennet in the lining of the skin sack, combined with the heat of the sun, caused the milk to separate into curds and whey. Croatian cheese in the sack was originally made from raw sheep's milk, without the addition of starter cultures. Since fermentation depended on the naturally present microflora coming from the milk or the environment, this could lead to differences in taste and aroma, consistency and microbial quality of the final product. Today, Croatian cheese in a sack is produced from sheep's, cow's and goat's milk or from their mixtures. Although the production technology has changed to some extent, the basic production parameters and the characteristics of the cheese have remained unique and recognisable [3,4].

The flavour of cheese, *i.e.* the combination of taste and odour, is one of the decisive criteria for consumer choice and acceptance. The flavour of freshly-made curds of various kinds of cheese is largely similar and is caused by the action of starter bacteria. During ripening, the characteristic flavour of cheese develops due to a complex series of biochemical reactions catalysed by living microorganisms or enzymes from several sources. The numerous compounds involved in cheese

flavour are mainly derived from three major metabolic pathways: (1) metabolism of residual lactose and of lactate and citrate, (2) lipolysis and fatty acid metabolism, and (3) proteolysis and amino acid catabolism [5, 6]. Cheese matured in an animal skin sack has a pronounced and piquant flavour. This characteristic flavour results from intense lipolysis and proteolysis as a consequence of the specific anaerobic conditions inside the skin sack, the autochthonous microorganisms from raw milk and skin, and manufacturing technology [7]. Although cheeses ripened in the animal skin have different production technologies and are produced from different types of milk, they are all characterised by high concentrations of long-chain fatty acids [8-11].

The concentration and composition of volatile compounds, often designated as aroma compounds, directly affect the flavour of the cheese. The aroma of most cheeses is characterised by the same classes of compounds. The differences between aroma compounds are more quantitative than qualitative. The aroma of most cheeses does not depend on the concentration of a particular compound itself, but on a critical balance or “weighted ratio” of all the components present (“component balance theory”) [12]. However, excessive concentrations of some compounds can cause off-flavours. Zabaleta, L. *et al.* reported that high proportions of acids lead to acid and/or rancid off-flavour [13]. It is generally recognized that the characteristic aroma of most cheeses results from the subtle combination of a large number of odorous volatile compounds present in the correct concentration ratios. Disturbance of this delicate balance can lead to off-flavours [14]. The same applies to the aroma of cheeses ripened in the animal skin. The volatile compounds responsible for the characteristic aroma of cheese ripened in the animal skin are usually esters, acids, methyl ketones, aldehydes, alcohols, sulphur compounds, and terpenes. Among them, acids, esters and alcohols usually predominate. As for the acids, short- and medium-chain fatty acids have a specific flavour and contribute directly to the cheese aroma, while long-chain fatty acids are the precursors of other flavour compounds and contribute indirectly to the cheese aroma. The esters are responsible for the fruity and floral notes, while the alcohols give the cheese a sharp aroma [7].

Croatian cheese in lambskin sack is only partially researched, and the studies mainly refer to cheese made from sheep’s milk. Kostelac, D. *et*

al. studied the composition of aroma compounds and sensory characteristics of Croatian sheep’s milk cheese in a sack produced with and without the addition of starter cultures during the ripening period [4]. Tudor Kalit, M. *et al.* studied Croatian cheese in a lambskin made from raw sheep’s milk to determine changes in composition, biochemical properties, and sensory characteristics during 60 days of ripening [8]. Lešić, T. *et al.* investigated the influences of the ripening period and different starter cultures on the basic chemical composition and fatty acid composition of autochthonous Croatian cow and sheep cheeses in a lambskin sack [15].

To the author’s knowledge, this is the first report on the volatile compound’s composition of Croatian cheese in a sack made from either raw cow’s or goat’s milk. The aim of this research was to investigate the volatile compounds of these traditional Croatian cheeses and relate them to their specific aroma. For this purpose, headspace solid-phase microextraction (HS-SPME) and ultrasonic solvent extraction (USE) were used in combination with gas chromatography-mass spectrometry (GC-MS).

Experimental

Materials

The solvents diethyl ether and pentane were purchased from Kemika (Zagreb, HRV) and distilled by careful fractional distillation before usage. Anhydrous sodium sulphate was purchased from Fluka Chemie (Buchs, CHE). The SPME fibres covered with DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) or PDMS/DVB (polydimethylsiloxane/divinylbenzene), were purchased from Supelco Co. (Bellefonte, PA, U.S.A.).

The cheeses in a sack made from goat or cow milk came from two family farms near Drniš, Croatia. The cheeses were made from raw, unpasteurised milk without the addition of dairy cultures and ripened in lambskin for 45 days. Samples of each cow’s and goat’s cheese was examined. All analyses were performed in triplicate, the results were calculated as mean (\bar{A}_v) \pm standard deviation (SD).

Headspace solid-phase microextraction of samples

HS-SPME was performed using manual SPME fibres, which were conditioned prior to use according to the manufacturer’s instructions by inserting them into the GC injector port. For HS-SPME extraction, 1 g of cheese was placed in a 15 mL amber glass vial and hermetically sealed with a PTFE/silicone septum.

The procedure applied to HS-SPME was described previously [16].

Ultrasonic solvent extraction of samples

USE was performed in an ultrasonic bath (Transsonic Type 310/H, DE) in the mode of indirect sonication, at the frequency of 35 kHz at $25\pm 3^\circ\text{C}$. Forty grams of the cheese samples were dissolved in 40 mL of distilled H_2O in a 250 mL flask. Anhydrous sodium sulphate (1.5 g) was added and the sample was extensively vortexed. A solvent mixture of pentane/diethyl ether 1:2 (v/v) was used for extraction. The procedure applied to USE was described previously [16].

Gas chromatography-mass spectrometry analysis

GC/MS analyses were performed on an Agilent Technologies GC/MS system (Palo Alto, CA, U.S.A.), GC model 7890A with a mass selective detector model 5975C, using nonpolar HP-5MS column (5% diphenyl and 95% dimethylpolysiloxane, $30\text{ m} \times 0.25\text{ mm i.d.}$, film thickness $0.2\ \mu\text{m}$). The GC operating conditions were the following: oven temperature was kept at 70°C for 2 min, then increased to 200°C at a rate of $3^\circ\text{C}/\text{min}$ and maintained at 200°C for 20 min. The carrier gas was helium with a flow rate of $1\ \text{mL}/\text{min}$, injector temperature 250°C , injection volume $1\ \mu\text{L}$, and split ratio 1:50. MS conditions were the following: ionization voltage $70\ \text{eV}$, ion source temperature 280°C , mass range 35-350 mass units. Individual peaks were identified by comparing their R_I (determined relative to the t_R of n -alkanes (C_9 - C_{25}) for the HP-5MS column) with those of authentic samples and literature values, as well as by comparing their mass spectra with the Wiley 275 MS library and the NIST17 mass spectral database. The percentage composition of the samples was calculated from the GC peak areas using the normalisation method (without correction factors).

Statistical analysis

Analysis of variance (one-way ANOVA) was used to assess the statistical difference between cow and goat cheese volatiles isolated by two isolation methods, HS-SPME and USE, using a significance level of $p \leq 0.05$.

Results and discussion

Isolation of volatile compounds from a complex mixture such as cheese to obtain representative extracts is very challenging. The flavour qualities of cheese are very much dependent on the volatile and semi-volatile organic compounds present in both the sample matrix and the headspace aroma. Therefore, in order to obtain a more complete insight into the

chemical composition of the cheese volatile compounds, two methods of isolation, HS-SPME and USE were used; both techniques have several advantages. The HS-SPME technique is sensitive, selective, fast, inexpensive, solvent-free, and easy to use. In the USE technique, the mechanical effect of ultrasound provides greater penetration of solvent into the matrix, *via* cavitation effects, and improves the extraction. The HS-SPME technique allows the isolation of the most volatile compounds from the headspace aroma, while the USE technique allows the isolation of less volatile compounds. The HS-SPME method enabled the identification of volatile compounds, such as short-chain linear fatty acids, alcohols, and esters, responsible for cheese aroma, while the USE method enabled the identification of semi-volatile long-chain fatty acids that do not directly contribute to cheese flavour but are important as precursors of other aroma compounds. One-way analysis of variance (ANOVA) was used to determine the presence or absence of significant differences ($p \leq 0.05$) between volatiles of cow and goat cheeses isolated with two methods of isolation, HS-SPME and USE. No significant differences ($p > 0.05$) in volatile composition among cheeses were observed.

Volatile compounds isolated by HS-SPME

HS-SPME was performed at a temperature of 50°C using two fibres of different polarity, a DVB/CAR/PDMS fibre and a PDMS/DVB fibre. According to the results of volatile compounds analysis, a total of 23 different volatiles were detected in the samples of cow and goat cheeses (Table 1). In both cheeses, carboxylic acids and alcohols were the dominant groups of volatile compounds.

A total of 21 volatile compounds were identified in the cow cheese samples, including 7 acids, 6 esters, 6 alcohols, 1 hydrocarbon and 1 ketone. The most abundant acids isolated on the DVB/CAR/PDMS fibre were acetic and hexanoic acids, while acetic and butanoic acids were the most abundant on the PDMS/DVB fibre. Propanoic acid, octanoic acid and 3-methylbutanoic acid were also detected on both fibres but at lower concentrations. The main alcohols isolated on the DVB/CAR/PDMS fibre were butan-2-ol and ethanol, while the most abundant alcohols isolated on the PDMS/DVB fibre were butan-2-ol and 3-methylbutan-1-ol. Other alcohols, found in lower amounts either on the DVB/CAR/PDMS or the DVB/PDMS fibre, were butane-2,3-diol, 2-phenylethanol and propan-1-ol. Ethyl hexanoate and ethyl octanoate were the main esters isolated on

DVB/CAR/PDMS fibre, while the main esters isolated on PDMS/DVB fibre were ethyl hexanoate, ethyl butanoate and ethyl 3-methylbutanoate. 3-Methylbutyl acetate was also detected in cow cheese, but only on the DVB/PDMS fibre. Butan-2-one was the only ketone, isolated on both fibres, and tetradecane was the only hydrocarbon, isolated only on the PDMS/DVB fibre (Table 1).

Seventeen compounds, including 5 acids, 5 esters, 4 alcohols, 2 ketones and 1 hydrocarbon were detected in the goat cheese samples. Hexanoic acid, octanoic acid, and butanoic acid were the most abundant acids isolated on the DVB/CAR/PDMS fibre, while hexanoic acid, butanoic acid and acetic acid were the most abundant on the PDMS/DVB fibre. 3-Methylbutanoic acid was also detected on both

fibres, but in a lower concentration. The main alcohols extracted from goat cheese on both fibres were butan-2-ol and ethanol. Other alcohols found in lower amounts were 3-methylbutan-1-ol, isolated on both fibres, and butane-2,3-diol, isolated only on the PDMS/DVB fibre. The most abundant esters isolated on the DVB/CAR/PDMS fibre were ethyl hexanoate and ethyl octanoate while the main esters isolated on the PDMS/DVB fibre were ethyl hexanoate, ethyl octanoate and ethyl butanoate. Ethyl decanoate, isolated on both fibres, and 3-methylbutyl acetate, isolated only on the PDMS/DVB fibre, were found in lower amounts. Butan-2-one and 3-hydroxybutan-2-one were the only ketones and 2,2,4,6,6-pentamethylheptane was the only hydrocarbon, all of which were isolated on both fibres.

Table 1

Volatiles of cow's and goat's cheeses matured in a sack isolated by HS-SPME.

No.	Compound name	RI HP-5MS	Peak area (%)±SD ^a I/II	
			cow	goat
<i>Acids</i>				
1.	Acetic acid	<900	22.6±0.07/10.6±0.07	5.3±0.01/6.3±0.14
2.	Propanoic acid	<900	3.4±0.03/2.2±0.04	-/-
3.	Butanoic acid	<900	2.9±0.10/8.8±0.06	6.1±0.01/6.7±0.14
4.	3-Methylbutanoic acid	<900	tr./3.2±0.02	1.0±0.01/0.6±0.07
5.	2-Methylbutanoic acid	<900	-/1.0±0.12	-/-
6.	Hexanoic acid	991	6.1±0.08/3.9±0.20	14.9±0.04/9.1±0.01
7.	Octanoic acid	1189	1.4±0.02/0.6±0.13	7.4±0.03/3.4±0.12
<i>Esters</i>				
8.	Ethyl butanoate	<900	-/3.4±0.09	1.5±0.03/2.9±0.04
9.	Ethyl 3-methylbutanoate	<900	-/3.0±0.18	-/-
10.	3-Methylbutyl acetate	<900	-/1.3±0.05	-/0.5±0.04
11.	Ethyl hexanoate	1008	4.0±0.14/3.8±0.05	10.7±0.09/9.4±0.13
12.	Ethyl octanoate	1207	3.2±0.11/1.0±0.09	7.3±0.02/3.3±0.07
13.	Ethyl decanoate	1406	tr./-	1.5±0.01/1.0±0.04
<i>Alcohols</i>				
14.	Ethanol	<900	14.9±0.02/4.9±0.03	10.0±0.14/7.1±0.13
15.	Propan-1-ol	<900	1.4±0.11/-	-/-
16.	Butan-2-ol	<900	19.3±0.11/10.1±0.06	18.7±0.06/19.7±0.21
17.	3-Methylbutan-1-ol	<900	0.8±0.06/6.1±0.05	2.3±0.01/1.7±0.02
18.	Butane-2,3-diol	<900	5.2±0.03/-	-/2.8±0.10
19.	2-Phenylethanol	1126	5.0±0.02/1.3±0.04	-/-
<i>Ketones</i>				
20.	Butan-2-one	<900	7.1±0.01/26.1±0.05	8.0±0.24/19.1±0.21
21.	3-Hydroxybutan-2-one	<900	-/-	0.9±0.10/1.6±0.02
<i>Hydrocarbons</i>				
22.	2,2,4,6,6-Pentamethylheptane	1000	-/-	1.1±0.04/1.2±0.03
23.	Tetradecane	1419	-/0.5±0.01	-/-
<i>Total identified (%)</i>			97.3/91.8	96.7/96.4

RI: retention index determined relative to a homologous series of *n*-alkanes (C₉ - C₂₅) on a HP-5MS column; SD - standard deviation (n = 3); ^a: average value (n = 3); I: DVB/CAR/PDMS fibre; II: PDMS/DVB fibre; - = not detected; tr. = traces < 0.1%.

The volatile compounds of Croatian cheese in a lambskin sack have not been sufficiently studied. There is only one paper on the volatile compounds of Croatian cheese in a sack, and it refers to sheep's milk cheese. In the paper of Kostelac, D. *et al.* isolation of cheese volatiles was performed using a manual SPME fibre coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) [4]. In the present research, the volatile composition of cow's or goat's cheese in a sack was investigated. The results have been compared with a sheep cheese sample prepared in the same way as our samples (without the addition of starter bacteria, 45 days of ripening, isolated on the same fibre). Regarding classes of compounds, the samples do not differ significantly in a qualitative way. The main volatile groups of compounds in sheep cheese were carboxylic acids, esters and alcohols, while in cow and goat cheeses the main volatile groups of compounds were carboxylic acids and alcohols. However, some classes of compounds, such as aldehydes and terpenes, were found in sheep cheese but not in cow and goat cheeses. There are also some qualitative and quantitative differences regarding the individual compounds in the cheese samples. For example, ethyl acetate, the major ester in sheep cheese, wasn't identified in cow and goat cheeses. Ethyl hexanoate and ethyl octanoate, the main esters in cow and goat cheese samples, were also identified in sheep cheese, but in lower amounts. Ethanol was the major alcohol in sheep cheese and one of the main alcohols in cow and goat cheeses, while butan-2-ol, the major alcohol in cow and goat cheeses, wasn't identified in sheep cheese. Acetic acid, butanoic acid, and hexanoic acid were the main acids in sheep cheese, which is in agreement with the results for cow and sheep cheeses. Octanoic acid, one of the main acids in goat cheese, was also identified in cow and sheep cheeses, but in lower amounts.

Most cheeses matured in animal skin are traditionally made from raw milk. Their flavour is the result of a combined action of non-starter lactic acid bacteria and other bacteria, yeasts and moulds that come from the raw milk, but also from the environment during cheese ripening. The cheese environment is characterized by a complexity of microbial populations which contribute to numerous biochemical reactions leading to the formation of flavour volatile compounds. The activity of a complex enzymatic system of microorganisms during cheese ripening under specific conditions (in the animal skin) gives the cheese specific sensory characteristics

[17-19]. The animal skin used as a ripening medium is composed of fibrous proteins such as collagen, elastin, reticulin, and keratin. This type of porous structure allows some permeability to air and water vapour, which affects the chemical and biochemical properties and the aroma compounds produced during ripening [8]. Some authors [20,21] reported that the ripening medium (goat skin or plastic barrel) affects the chemical composition of Tulum cheese. In general, the samples contained the same volatile aroma compounds, but their concentrations were different. The studies of the natural microbial population of cheeses ripened in the animal skin have shown that the lactic acid bacteria of the genera *Lactococcus*, *Enterococcus*, *Lactobacillus* and *Streptococcus* dominate, as well as yeasts and moulds. The microbiota in traditional cheeses has the capacity to degrade milk fat through lipolysis. The result of lipolysis is free fatty acids of various sizes, of which the short-chain and medium-chain free fatty acids contribute directly to the flavour of the cheese [17,18,22].

Although the predominant volatile compounds responsible for the characteristic aroma of cheese ripened in the animal skin are usually esters, acids, methyl ketones, aldehydes, alcohols, sulphur compounds, and terpenes, the volatile profile is specific to each type of cheese, which is explained by the different production technologies, type of milk, and ripening conditions [7].

Free fatty acids make an important contribution to cheese aroma, either directly through their aroma notes (especially short- and medium-chain fatty acids) or as precursors of carbonyl compounds, alcohols, alkanes and esters. Fatty acids with long-chain carbon atoms are usually formed by lipolysis of triglycerides, those with medium-chain carbon atoms by the degradation of lactose and amino acids, and short-chain fatty acids can also be formed by the oxidation of aldehydes, ketones and esters [23-25]. Short-chain linear fatty acids dominated in both cow and goat studied cheeses. Free fatty acids with 4-12 carbon atoms produce specific flavours, *e.g.*, hexanoic acid (caproic acid) and octanoic acid (caprylic acid) are responsible for goat, waxy, and cheese flavours [26], butanoic acid (butyric acid) for rancid, cheesy flavours [27], and propanoic acid for vinegar and pungent flavours [28]. Acetic acid also imparts a sharp and vinegary flavour to cheese [7], while branched-chain fatty acids, 3-methylbutanoic acid and 2-methylbutanoic acid, are associated with the sweaty and rancid flavour of cheese [29].

Alcohols are common cheese volatiles that impart the flavours of alcohol, wine, sweetness and fruits to cheese and give a sharp aroma to cheese [30]. Many metabolic pathways are involved in the biosynthesis of the alcohols found in cheese: lactose metabolism, methyl ketone reduction, amino acid metabolism as well as degradation of linoleic and linolenic acid [23]. Lactose metabolism leads to the formation of ethanol *via* the pentose phosphate pathway and the mixed acid pathway, and not only ethanol but also butane-2,3-diol is formed [23]. The secondary alcohol butan-2-ol is mainly derived from citrate metabolism [21]. According to Ferreira, I.M. *et al.*, ethanol and butan-2-ol contribute to the formation of alcoholic notes in cheese, propan-1-ol to alcohol and sweet notes, and butane-2,3-diol to fruity notes [28]. In addition, ethanol is an important precursor in the formation of esters. Santamarina-García, G. *et al.* reported low odour impact ratio values (OIR<1) of alcohols in Idiazabal cheese. Butan-2-ol, which was one of the predominant alcohols, was the only odour-active alcohol in this raw milk-derived cheese. These results confirmed the small contribution of alcohols to cheese aroma, mainly due to their high detection thresholds [31]. The branched-chain alcohol 3-methylbutan-1-ol is formed during the reduction of the aldehyde produced from leucine [32] and provides a fruity-floral note [33]. 2-Phenylethanol, which is probably produced from phenylalanine, is one of the most fragrant aromatic alcohols with pleasant notes of roses, violets, and flowers [32].

Esters in cheese are responsible for the sweet, fruity (especially ethyl esters), floral notes at low concentrations, and yeasty notes at high concentrations. In general, they are known to have a low perception threshold [1]. In addition, these compounds can contribute to the aroma of cheese by minimizing the sharpness and bitterness of fatty acids and amines, respectively [32]. The biosynthesis of esters occurs through two enzymatic mechanisms: esterification and alcoholises. Esterification is the formation of esters from alcohols and carboxylic acids, whereas alcoholises is the production of esters from alcohols and acylglycerols or from alcohols or acyl-coenzyme A [34]. According to Liu, S.Q. *et al.*, ethyl butanoate contributes to the formation of apple, banana, sweet, and fruity notes; ethyl hexanoate to the formation of banana, pineapple, and wine notes; ethyl octanoate to the formation of pear, pineapple, apricot, and floral notes; and ethyl decanoate to the formation of apple, brandy,

grape, fruity, and oily notes [34]. Curioni, P.M.G. and Bosset, J.O. reported that 3-methylbutyl acetate is responsible for fruit and banana notes in cheese, while ethyl 3-methylbutanoate is responsible for notes of fresh cheese [32].

Ketones, which have a typical odour and low perception threshold, are the products of lipid degradation produced by β -oxidation and decarboxylation of fatty acids [1]. Butan-2-one and 3-hydroxybutan-2-one (acetoin), likely derived from butane-2,3-dione (diacetyl), are responsible for the buttery and sour lactic aroma in many kinds of cheese [1,12,35].

Hydrocarbons have high detection thresholds and therefore their contribution to cheese aroma is much less than other volatile components [27]. One of the most common hydrocarbons found in cheeses that ripen in animal skin is 2,2,4,6,6-pentamethylheptane [7].

Volatile and semi-volatile compounds isolated by USE

Sixteen different volatile compounds, including 9 acids, 4 esters, 2 carbonyl compounds, and 1 alcohol were isolated by USE (Table 2). Fatty acids were predominant volatiles, accounting for 78.2% of the total cow cheese sample and 93.5% of the goat cheese sample. For comparison, in cheese extracts isolated by HS-SPME, fatty acids accounted for 36.4% (DVB/CAR/PDMS) and 30.3% (PDMS/DVB) of the total sample of cow's milk cheese and 34.7% (DVB/CAR/PDMS) and 26.1% (PDMS/DVB) of the total sample of goat's cheese. (Z)-Octadec-9-enoic acid (oleic acid), hexadecanoic acid (palmitic acid) and decanoic acid (capric acid) were the most represented in both cheeses. Dodecanoic acid (lauric acid) and tetradecanoic acid (myristic acid) were detected in both cheeses, but in lower amounts. All of the long-chain acids mentioned were not identified by HS-SPME. It is considered that long-chain fatty acids, due to their high perception threshold values, do not have a significant impact on the aroma of cheese [23]. Tudor Kalit, M. *et al.* [8] reported that palmitic, oleic, and stearic acids are the predominant free fatty acids in Croatian sheep cheese matured in a lambskin sack, which is in accordance with the research results reported by Lešić, T. *et al.* [15] on the fatty acid composition of Croatian cow and sheep cheeses matured in lambskin sacks.

In this study, USE in combination with GC-MS analysis is proved to be a simple and fast method for the isolation and identification of long-chain fatty acids without their prior derivatization.

Table 2

Volatiles of cow's and goat's cheeses matured in a sack isolated by USE.				
No.	Compound name	RI, HP-5MS	Peak area (%)±SD ^a I/II	
			cow	goat
<i>Acids</i>				
1.	3-Methylbutanoic acid	<900	1.9±0.21	0.4±0.02
2.	2-Methylbutanoic acid	<900	0.5±0.02	-
3.	Hexanoic acid	991	7.0±0.13	3.6±0.06
4.	Octanoic acid	1189	11.8±0.21	7.0±0.11
5.	Decanoic acid	1381	15.7±0.04	25.6±0.17
6.	Dodecanoic acid	1590	2.1±0.04	3.6±0.03
7.	Tetradecanoic acid	1796	5.7±0.03	5.7±0.20
8.	Hexadecanoic acid	1995	16.7±0.09	21.4±0.03
9.	(Z)-Octadec-9-enoic acid	2176	16.8±0.07	26.2±0.24
<i>Esters</i>				
10.	Ethyl hexanoate	1008	tr.	-
11.	Ethyl octanoate	1207	tr.	-
12.	δ-Dodecalactone	1719	3.4±0.01	-
13.	Ethyl tetradecanoate	1804	1.1±0.05	0.5±0.01
<i>Alcohols</i>				
14.	2-Phenylethanol	1126	4.1±0.14	-
<i>Carbonyl compounds</i>				
15.	Tetradecanal	1625	-	1.7±0.03
16.	Pentadecan-2-one	1708	1.7±0.06	-
Total identified (%)			88.5	95.7

RI: Retention index determined relative to a homologous series of *n*-alkanes (C₉ - C₂₅) on a HP-5MS column; SD - standard deviation (n = 3); ^a: average value (n = 3); - = not detected; tr. = traces < 0.1%.

Conclusions

The presented research has shown by means of HS-SPME analysis that carboxylic acids and alcohols were the dominant volatile compounds in both cheeses. Short-chain linear fatty acids dominated in both cheeses studied. Acetic, butanoic, and hexanoic acids were the major acids in cow cheese, while hexanoic, butanoic, acetic, and octanoic acids were the major acids in goat cheese. These acids produce specific flavours, e.g., hexanoic and octanoic acids are responsible for goat, waxy, and cheesy flavours, butanoic acid for rancid, cheesy flavours, propanoic and acetic acids for sharp, vinegary and pungent flavours. Butan-2-ol and ethanol were the major alcohols in goat cheese, while butan-2-ol, ethanol and 3-methylbutan-1-ol were the major alcohols in cow cheese. Ethanol and butan-2-ol contribute to the formation of alcoholic notes in cheese. In addition, ethanol is an important precursor in the formation of esters. The branched-chain alcohol 3-methylbutan-1-ol provides a fruity-floral note.

Long-chain fatty acids, namely (Z)-octadec-9-enoic, hexadecanoic and decanoic acid, were the most abundant compounds in both cheeses isolated by USE. These acids do not contribute directly to cheese flavour but are important as precursors to other aroma compounds. According to this study, USE in combination with GC-MS

analysis enabled the isolation and identification of long-chain fatty acids without their prior derivatization.

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