



J. Serb. Chem. Soc. 79 (8) 927–939 (2014)
JSCS–4637

Characterization of volatiles in Beaten cheeses “Bieno sirenje” by SPME/GC-MS: Influence of geographical origin

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(Received 17 September, revised 17 November, accepted 18 November 2013)

Abstract: In this study, the volatile profiles of an economically important type of cheeses for the FYR Macedonian dairy sector were characterized. A total of eighteen samples belonging to 6 different geographical regions of Beaten cheese, including cheeses from Kumanovo, Tetovo, Struga, Resen, Veles and Radoviš, were comparatively studied for their volatile profiles. Sixty one volatile compounds were identified in the cheeses by solid-phase micro-extraction combined with gas chromatography–mass spectrometry. The results are discussed based on their chemical classes (17 esters, 9 ketones, 10 acids, 8 alcohols, 6 terpenes and 11 miscellaneous compounds). Acids, esters and alcohols were the most abundant classes identified and were highly dependent on the geographical origin of the cheeses. Beaten cheese from Struga had the highest levels of carboxylic acids, ketones, alcohols, esters and terpenes. The Beaten cheese of other geographical origin had low levels of volatiles, probably due to the effects of the variable characteristics of the employed milk and differences in the cheese making processes, which affected the biochemical processes. The results suggested that each cheese from different geographical regions had a different volatiles profile and that the manufacturing technique and the ripening stage of the cheeses played major roles in determining the distribution of the volatile compounds.

Keywords: Beaten cheese; geographical regions; volatile compounds; SPME; GC–MS.

INTRODUCTION

Several reviews summarize the present knowledge of volatile formation, profiling in different types of cheese and the analytical techniques for their study.^{1,2} The typical flavour of each cheese variety is determined by the presence of volatile compounds and their relative concentrations. Although over 600 vola-

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doi: 10.2298/JSC130917135S

tile compounds have been identified in cheeses, only a small fraction can be considered as “flavour impact” compounds. The most potent flavour compounds in cheeses are aldehydes, alcohols, carboxylic acids and esters derived from the catabolism of amino acids. The main agents related to flavour formation in cheese are indigenous milk enzymes, rennet and microbial enzymes from the wild microbial flora and/or commercial starters or adjunct cultures used.

For some cheese varieties, a specific compound group is recognized as being the major contributor to flavour. In general, raw milk cheeses contain a more diverse group of volatile compounds than cheeses made from pasteurized milk, as pasteurization of milk promotes changes that affect the flavour. In hard Italian cheese varieties, free fatty acids (FFAs) are the important contributors, while in blue-veined ripened cheeses, the impact of FFAs on the flavour is less owing to the dominant influence of methyl ketones. The volatile chromatographic pattern was used in the classification of some cheese varieties.³ The formation of these compounds in cheese results from proteolysis, lipolysis and the metabolism of lactose and lactate.⁴

Beaten cheese is an autochthonous product in the FYR Macedonia, which originates from the territory of Mariovo where it is manufactured on the pasture land exclusively from sheep's milk. It is characterized by extremely salty flavour and a firm texture with visible holes (eye). This kind of cheese is named after the manufacturing process: the cheese curd is beaten; therefore, it is called “Beaten cheese” or “*Bieno sirenje*”. Beaten cheeses are produced almost exclusively on state territory mainly from raw milk of cows, ewes and goats. The production of cheese varies in different regions, which results in the appearance of different cheese varieties. The characteristics of the milk used for cheese production are also variable and depend on the geographical region, season of milking and the nutrition method. Following coagulation of the milk at 34 °C for 45 min using animal rennet, the curd is stirred and beaten manually or mechanically until a homogenous compact structure is obtained. After drainage, the curd is cooked in a hot water at 70 to 90 °C for 20 min. Following the cooking, the curd is beaten for 5 to 10 min. The curd is then moulded and transferred to a cotton cloth for hanging for about 24 h. Then the curd is pre-ripened for 3 days. During this period, the curd gains a specific yellowish colour, a hard texture and visible holes. The cheese loafs are cut in slices 30 to 40 cm long and 4 to 5 cm wide. The cheese blocks are salted by dry-salting for 24 h and then brine salting. Finally the pieces are packed in plastic barrels containing brine with concentration of 18 to 20 % (w/V) NaCl for long-term storage. Such differences in manufacture procedure have strong influence on the characteristics and appearance of the cheese varieties.⁵

The aim of this study was to determine and compare the volatile compounds of Beaten cheeses produced in different geographical regions. Due to the lack of

information on such cheeses, the results should provide a better understanding and notification of these cheeses, which would lead to a protected designation.

EXPERIMENTAL

Cheese samples

Eighteen samples from 6 different regions of Beaten cheese (three samples per region) were collected from FYR Macedonia in 2012. The ages of the cheeses were between 1–9 months old (Table I). As far as possible, ripened or ready for sale cheese samples were collected. Production dates were noted when known; however, some samples were produced by artisanal methods without packaging and labelling. Thus, to assess the production dates for these, the manufacturer's declarations were taken into account. The cheese samples were stored in sterile plastic bags and transported to the laboratory of the Food Engineering Department, Canakkale Onsekiz Mart University, Turkey. The cheese samples were vacuum-packed and stored at -20°C until analysis, which was performed within 1 month of sample collection.

TABLE I. Short summary of the production characteristics of the analysed cheeses

Characteristic	Geographical origin of Beaten cheeses ($n = 18$)					
	Kumanovo	Tetovo	Struga	Resen	Veles	Radoviš
Milk type	Ewe/Goat	Ewe/Cow	Ewe	Cow/Ewe	Cow	Ewe/Goat
Pasteurisation	No	No	No	Yes	Yes	No
Rennet type	Home made	Home made	Chymosin	Chymosin	Chymosin	Home made
Cooking temperature, $^{\circ}\text{C}$	80	90	80	70	70	60
Ripening type	Under brine	Under brine	Under brine	Vacuum packed	Vacuum packed	Under brine or dry
Ripening period	> 9 months	8–9 months	> 9 months	< 2 months	< 1 months	9 months
Industrialised	No	No	No	Yes	Yes	No

SPME GC/MS

The volatile compounds were isolated from Beaten cheese by solid-phase microextraction (SPME) methods. For this purpose, 5 g of grated and homogenized cheese sample was placed in a 40-mL amber-coloured SPME vial and then 1 g of NaCl was added and 10 μL of internal standard (0.8 μg 2-methyl-3-heptanone for basic/neutral compounds and 55.2 μg 2-methylvaleric acid for acidic compounds). The vial was vortexed for one min, and held in a water bath for 20 min at 40°C . Volatile compounds were extracted by placing a SPME fibre (2 cm – 50/30 μm divinylbenzene (DVB)/Carboxen/polydimethylsiloxane (PDMS), Supelco) into a vial and exposing it to a head-space (HS) vial for 20 min at 40°C . The volatile compounds were determined using a HS-GC–MS system, which consisted of a HP 6890 GC and a 7895C mass selective detector (Agilent Technologies, Wilmington, DE, USA). Helium was used as the carrier gas. The total flow was 1.2 mL min^{-1} . The GC was fitted with a HP5 MS column (30 m $\times$ 0.25 mm id $\times$ 0.25- μm film thickness, J and W Scientific, Folsom, CA, USA). The mass spectra were recorded in the electron impact mode at an ionization voltage of 70 eV in the 33–300 a.m.u. mass range. Oven temperature was programmed from 40 to 230°C at rate of $10^{\circ}\text{C min}^{-1}$, with initial and final hold times of 5 and 20 min, respectively. The volatiles were tentatively identified by comparison of the mass spectra of the unknown

compounds with those in the National Institute of Standards and Technology (NIST 08) and Wiley Registry of Mass Spectral Data, 7th edition (Wiley 05) mass spectral databases. The retention indices were calculated using an *n*-alkane series.⁶ Quantification of flavour compounds were realised from the relative abundances of the volatiles compounds positively using Eq. (1):⁷

$$\text{Mean abundance } (\mu\text{g/kg}) = c_{\text{IS}} \times A_{\text{C}} / A_{\text{IS}} \quad (1)$$

where, c_{IS} is concentration of internal standard (lg/kg), A_{C} is peak area of compound, A_{IS} is peak area of the internal standard.

Statistical analysis

Data from GC-MS measurements were analyzed using one-way analysis of variance (ANOVA). The HSD Tukey's test was applied to compare the mean values of the volatile compounds. These statistical treatments were performed using the SPSS program for Windows, version 9.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Volatile compounds

A total of 62 volatile compounds were identified in Beaten cheeses from 6 different geographical regions. These compounds were grouped into chemical classes including 10 acids, 8 alcohols, 17 esters, 9 ketones, 6 terpenes and 11 miscellaneous compounds. Duplicate analyses for all samples were performed and the average values with standard deviation (*SD*) are listed in Tables II–VII. The volatiles of Beaten cheeses have not hitherto been characterized and the results are discussed by comparison to data reported by other researchers for different cheeses.

Carboxylic acids

Carboxylic acids were the most abundant chemical family isolated from the headspace of the Beaten cheeses from the different regions. A high concentration ($173624.6 \mu\text{g } 100 \text{ g}^{-1}$) of these acids was detected in the Struga cheeses. A low concentration ($1863.6 \mu\text{g } 100 \text{ g}^{-1}$) was detected in the samples of Resen cheeses. Five different acids were identified in the Kumanovo and Resen Beaten cheeses, and their concentrations were 13347.2 and $1863.6 \mu\text{g } 100 \text{ g}^{-1}$, respectively. Six different acids were identified in the Tetovo and Radoviš Beaten cheeses, and their concentration were 4220.8 and $13773.8 \mu\text{g } 100 \text{ g}^{-1}$, respectively. Acetic acid was identified in the Veles Beaten cheese ($371.9 \mu\text{g } 100 \text{ g}^{-1}$), while considerably higher levels of butanoic ($59993.8 \mu\text{g } 100 \text{ g}^{-1}$) and hexanoic acid ($84803.1 \mu\text{g } 100 \text{ g}^{-1}$) were detected in the Struga cheeses. Throughout the ripening period of the cheeses, most of carboxylic acids came from lipolysis of triglycerides followed by those produced from lactate metabolism; therefore, lipolysis was the main pathway responsible for the release of carboxylic acids in Beaten cheeses. Acetic acid was probably the product of citrate or lactate fermentation of amino acid catabolism by bacteria.

TABLE II. Contents of acids in the Beaten cheeses, $\mu\text{g } 100 \text{ g}^{-1}$ ($n = 18$); * – significant statistical differences (Tukey's test, $P < 0.05$); n.q.: not quantified; NS – not significant; RI – retention index

Compound	RI	Geographical origin						P value
		Kumanovo ($n = 3$)	Tetovo ($n = 3$)	Struga ($n = 3$)	Resen ($n = 3$)	Veles ($n = 3$)	Radoviš ($n = 3$)	
Acetic acid	614	n.q.	n.q.	n.q.	n.q.	371.9 $\pm$ 71.2	n.q.	*
Pentanoic acid	882	n.q.	1088.9. $\pm$ 47.3	928.8 $\pm$ 13.9	188.5 $\pm$ 52.3	0.7 $\pm$ 0.06	437.5 $\pm$ 80.0	*
2-Methylbutanoic acid	889	n.q.	361.2 $\pm$ 420.5	n.q.	0.6 $\pm$ 0.1	0.6 $\pm$ 0.07	n.q.	NS
Butanoic acid	890	7493.6 $\pm$ 3141.7	1137.8 $\pm$ 8.5	59993.8 $\pm$ 1509.1	501.5 $\pm$ 22.1	1090.1 $\pm$ 37.7	12850.2 $\pm$ 1409.9	*
3-Methylbutanoic acid	909	n.q.	n.q.	n.q.	n.q.	n.q.	41.9 $\pm$ 4.9	*
Hexanoic acid	1060	3253.4 $\pm$ 1344.4	n.q.	84803.1 $\pm$ 2095.1	23.9 $\pm$ 9.5	489.7 $\pm$ 19.1	263.1 $\pm$ 28.8	*
Octanoic acid	1204	169.5 $\pm$ 54.6	1259.1 $\pm$ 485.3	1981.6 $\pm$ 37.5	819.4 $\pm$ 259.4	191.4 $\pm$ 16.6	175.9 $\pm$ 8.5	*
Nonanoic acid	1276	6.8 $\pm$ 0.04	n.q.	665 $\pm$ 54.1	n.q.	n.q.	n.q.	*
Decanoic acid	1379	2423.6 $\pm$ 1007.2	373.8 $\pm$ 179.3	25234.4 $\pm$ 3261.4	330.02 $\pm$ 210.3	42.3 $\pm$ 5.9	5.1 $\pm$ 0.6	*
Dodecanoic acid	1556	n.q.	n.q.	17.8 $\pm$ 1.2	n.q.	n.q.	n.q.	*
Total acids		13347.1	4220.8	173624.6	1863.6	2186.8	13773.8	

The differences between cheeses in the volatile acid concentrations are associated with the maturation periods since the longer maturation period results in higher amount of carboxylic acids (Tables I and II). The variabilities in the milk composition during the seasonal production of the used milk could also impact these differences.⁸ Acid compounds constituted 97 % of the volatile compounds isolated from the Beaten cheeses. Significant differences ($P < 0.05$) for all acids were found, except for 2-methylbutanoic acid. Other carboxylic acids detected in Beaten cheese are listed in Table II.

Hexanoic acid, a short-chain carboxylic acid, contributes to the typical aroma of Struga Beaten cheese. The fatty acids hexanoic, octanoic and decanoic acids were widely recognized as being responsible for the characteristic aroma of goat cheeses, giving rise to the trivial terms caproic, caprylic and capric acids, respectively,⁹ and their contribution to the volatile profile of Beaten cheeses has been showed in this study as well.

Acetic and propanoic acids were associated with the slight sour taste of Veles Beaten cheeses. Branched-chain fatty acids (BCFA) were not characteristic active compounds of Beaten ewe's cheeses in general. 2-Methylbutanoic acid was the most abundant acid found in Tetovo Beaten cheese and it provides a rancid cheese and sweaty odours.¹⁰ In this case, as raw sheep milk was used for

some cheese manufacture, the native microorganisms of non-pasteurized milk may have a significant contribution on its final volatile profile.¹¹

Ketones

Most of the ketones in Beaten cheeses were methyl ketones (Table III). There were significant differences among the cheeses ($P < 0.05$) from different origin. The highest ($755.5 \mu\text{g } 100 \text{ g}^{-1}$) and lowest ($27.5 \mu\text{g } 100 \text{ g}^{-1}$) concentration were identified in Kumanovo and Radoviš Beaten cheeses, respectively. Methyl ketones are produced from free fatty acids by an alternative pathway to β -oxidation.¹¹ About 7 ketones were identified in Kumanovo Beaten cheese and the highest concentration of 2-heptanone was detected in Kumanova Beaten cheese. In addition, some identified ketones, including 2-octanone, 8-nonen-2-one and 2-nonanone were rarely found in cheeses from other regions. 2-Butanone, with a butterscotch odour, was identified as the main odorant in Cheddar cheese¹² and 2-heptanone, with a herbaceous odour, is an important flavour compound of Emmentaler and natural creamy Gorgonzola cheeses.¹³ Fruity, floral and musty notes are associated with various methyl ketones, such as 2-octanone, 2-nonanone and 2-undecanone, so the presence of these volatile compounds could be considered beneficial to the flavour of cheese.¹⁴ 2-Butanone

TABLE III. Contents of ketones in the Beaten cheeses, $\mu\text{g } 100 \text{ g}^{-1}$ ($n = 18$); * – significant statistical differences (Tukey's test, $P < 0.05$); n.q.: not quantified; NS – not significant; *RI* – retention index

Compound	<i>RI</i>	Geographical origin						<i>P</i> value
		Kumanovo ($n = 3$)	Tetovo ($n = 3$)	Struga ($n = 3$)	Resen ($n = 3$)	Veles ($n = 3$)	Radoviš ($n = 3$)	
Acetone	<600	n.q.	14.6 ± 0.5	n.q.	n.q.	n.q.	n.q.	*
2-Butanone	612	n.q.	n.q.	385.2 ± 1.6	n.q.	n.q.	n.q.	*
2-Pentanone	684	92.2 ± 6.3	4.7 ± 1.6	231.5 ± 1.5	n.q.	5.4 ± 0.2	n.q.	*
3-Hydroxy-2-butanone	711	n.q.	448.2 ± 37.1	n.q.	100 ± 25.8	115.3 ± 18.4	26.5 ± 4.1	*
2-Hexanone	790	16 ± 1.7	n.q.	16.7 ± 2	n.q.	n.q.	n.q.	*
2-Heptanone	896	520 ± 50.9	6.3 ± 5.9	62.1 ± 3.7	32.5 ± 0.7	24.7 ± 6.5	n.q.	*
2-Octanone	991	12.2 ± 2.8	–	0.6 ± 0.1	n.q.	n.q.	n.q.	*
8-Nonen-2-one	1088	11.2 ± 1.2	n.q.	0.05 ± 0	n.q.	n.q.	n.q.	*
2-Nonanone	1094	103.9 ± 10.2	1.8 ± 2.2	0.06 ± 0	8.3 ± 1.8	6.2 ± 2	1 ± 0.2	*
Total ketones		755.5	475.6	796.0	140.9	151.5	27.4	

and 2-pentanone were the main ketones in Struga Beaten cheese, similar to Malatya cheeses made from raw milk.¹⁵ Resen and Veles Beaten cheeses were differentiated from the other cheeses due to their similar ketone concentration of 140.9 and 151.5 $\mu\text{g } 100 \text{ g}^{-1}$, respectively. On the other hand, 2-butanone was identified only in Struga Beaten cheese. Seven different types of ketones were identified in Tetovo Beaten cheese with the concentrations of 3-hydroxy-2-butanone or acetoin (448.2 $\mu\text{g } 100 \text{ g}^{-1}$) and acetone (14.6 $\mu\text{g } 100 \text{ g}^{-1}$) being the greatest (Table III). Veles Beaten cheese was also rich in ketones (7 types) with acetoin (115.3 $\mu\text{g } 100 \text{ g}^{-1}$) and 2-heptanone (24.7 $\mu\text{g } 100 \text{ g}^{-1}$) being predominant, while the others were present at considerably lower levels (0.2–6.2 $\mu\text{g } 100 \text{ g}^{-1}$). The ketones followed a similar trend as was described for the acids. However, the reduction in the concentration of ketones was higher in cheese from the Radoviš region. 2-Pentanone and 2-heptanone were previously identified as the prevailing ketones in the volatile fraction of Parmigiano,¹⁶ whereas acetoin was identified at higher concentrations in pasteurized milk cheeses than in raw milk cheeses.¹⁷ Similar results were found in Spanish regional raw milk cheeses with protected designation of origin (PDO).¹⁸ Therefore, they could play an important role in the final aroma of these cheeses made from raw milk.

Alcohols

Eight different alcohols consisting of primary, secondary, and branched-chain alcohols were present in the cheese samples. The levels of the alcohols were significantly affected ($P < 0.05$) by the different methods of cheese manufacture. Generally, primary alcohols originate from the corresponding aldehydes produced from fatty acids and from amino acid metabolism. Among these, ethanol may be formed by lactose metabolism or by reduction of acetaldehyde.¹⁶ Secondary alcohols are obtained by the enzymatic reduction of methyl ketones.⁴ Alcohols were quantitatively the most abundant volatiles in Kumanovo Beaten cheese (Table IV) with 7 different alcohols being identified in this cheese. 2-Heptanol, 2-pentanol and benzyl alcohol were the most abundant alcohols in the cheeses from Kumanovo (Table IV). The concentration of alcohols in the cheeses from other regions may have been higher than the reported levels as it was reported that the level of alcohols can fluctuate during ripening.¹⁹ Thus, at the time of analysis, the cheeses examined in the present study were not sampled at the time where the levels of the various alcohols were at their maximal. Similar concentrations of total alcohols were isolated in Tetovo (74.1 $\mu\text{g } 100 \text{ g}^{-1}$) and Struga (88.6 $\mu\text{g } 100 \text{ g}^{-1}$) Beaten cheeses, although the predominant alcohols differed between these cheeses. 2-Pentanol was detected in the Struga Beaten cheeses, while, on the other hand, 1-butanol was detected in the Tetovo Beaten cheese. Two alcohols were identified in Resen and Veles Beaten cheeses with the

main one being 3-methyl-1-butanol. Radoviš Beaten cheese did not contain high levels of alcohols, however, the concentration of alcohols was similar with the Veles (9.9 $\mu\text{g } 100 \text{ g}^{-1}$) Beaten cheese.

TABLE IV. Contents of alcohols in the Beaten cheeses, $\mu\text{g } 100 \text{ g}^{-1}$ ($n = 18$); * – significant statistical differences (Tukey's test, $P < 0.05$); n.q.: not quantified; *RI* – retention index

Compound	Geographical origin							<i>P</i> value
	<i>RI</i>	Kumanovo ($n = 3$)	Tetovo ($n = 3$)	Struga ($n = 3$)	Resen ($n = 3$)	Veles ($n = 3$)	Radoviš ($n = 3$)	
2-Pentanol	701	249.6 ± 28.9	n.q.	83.3 ± 1.7	n.q.	n.q.	n.q.	*
1-Butanol	729	99.6 ± 12.9	71.7 ± 18.2	n.q.	n.q.	n.q.	4.1 ± 0.7	*
3-Methyl-1-butanol	730	n.q.	n.q.	5.1 ± 0.07	180.4 ± 44.9	8.2 ± 0.8	3.2 ± 0.6	*
2-Heptanol	914	277.8 ± 35.2	n.q.	n.q.	n.q.	1.5 ± 0.1	n.q.	*
3-(Methylthio)-1-propanol	983	1.04 ± 0.2	n.q.	n.q.	n.q.	n.q.	n.q.	*
Benzyl alcohol	1039	161.1 ± 32.3	n.q.	n.q.	n.q.	n.q.	0.1 ± 0.01	*
2-Nonanol	1104	19.9 ± 1.2	n.q.	n.q.	n.q.	n.q.	0.01 ± 0	*
Benzenethanol	1118	17.2 ± 0.5	2.4 ± 0.8	n.q.	n.q.	n.q.	2.9 ± 0.5	*
Total alcohols		826.4	74.1	88.5	180.7	9.8	10.4	

2-Heptanol was the highest secondary alcohol isolated in the artisanal Kumanovo cheese, which was previously identified as a key odorant of Gorgonzola and Grana Padano cheeses,¹³ and was detected in the highest concentrations in semi-hard Spanish goat cheeses.²⁰ 2-Pentanol has lower detection thresholds,¹⁸ which plays an important role in the aroma of ewe raw milk La Serena cheese. The present results indicated that 2-butanol was the most abundant compound, followed by 3-methyl-1-butanol, 1-propanol and ethanol. Similar results were observed in the case of the aromatic fraction of other raw milk cheeses, in which all these components were found in high amounts.¹⁸ The large amount of 2-butanol was formed by the reduction of 2,3-butanedione to 2-butanol during ripening, due to the high activity of non-starter lactic acid bacteria.²¹

Esters

These compounds are produced by enzymatic or chemical reactions of fatty acids with primary alcohols, so the alcohol concentration is a limiting factor in ester production.²² Seventeen esters were found in the 6 Beaten cheeses of different geographical origin and the most frequently identified sub-groups were seven

ethyl, four methyl, two isopropyl, two isobutyl, one isoamyl and one methylbutyl esters (Table V). The amounts of esters were significantly different ($P < 0.05$) in the cheeses, except for octanoic acid methyl ester. Esters were the unique chemical family with a high presence in Radoviš Beaten cheese because these cheeses had the longest maturation period (Table V). This increase could be due to the esterification of acids and alcohols. The ethyl esters of hexanoic acid and butanoic acid were the most abundant esters isolated in the Kumanovo and Resen Beaten cheese. These esters were obtained from esterification reactions occurring with hexanoic and butanoic acids, respectively. The ethyl esters of butanoic and hexanoic acids were identified as two of the most potent odorants of Cheddar, Emmentaler, creamy Gorgonzola, Grana Padano and Pecorino cheeses.¹³ These two esters, in addition to ethyl acetate, were the major esters identified in

TABLE V. Contents of esters in the Beaten cheeses, $\mu\text{g } 100 \text{ g}^{-1}$ ($n = 18$); * – significant statistical differences (Tukey's test, $P < 0.05$); n.q.: not quantified; NS – not significant; RI – retention index

Compound	RI	Geographical origin						<i>P</i> value
		Kumanovo ($n = 3$)	Tetovo ($n = 3$)	Struga ($n = 3$)	Resen ($n = 3$)	Veles ($n = 3$)	Radoviš ($n = 3$)	
Ethyl acetate	603	126.3±16.3	n.q.	n.q.	n.q.	n.q.	n.q.	*
Methyl butanoate	716	n.q.	n.q.	n.q.	3.8 ±0.7	n.q.	n.q.	*
Ethyl butanoate	804	9.1±0.8	156.5 ±35.9	65.3 ±1.4	4.6 ±1	2.7 ±0.3	n.q.	*
Methyl hexanoate	927	4.5±0.2	n.q.	10.2 ±1.7	0.8 ±0.2	0.6 ±0.1	n.q.	*
Isobutyl butanoate	959	13.4±1.7	n.q.	n.q.	n.q.	n.q.	n.q.	*
Ethyl hexanoate	1006	287.8±33.7	120.6 ±52.4	8.7 ±0.7	n.q.	n.q.	n.q.	*
Isoamyl butyrate	1058	1.4±0.2	n.q.	n.q.	n.q.	n.q.	n.q.	*
Methyl octanoate	1124	2.4±2.6	7.5 ±6	8.3 ±0.6	n.q.	n.q.	n.q.	NS
Isobutyl hexanoate	1136	2.2±0.2	n.q.	n.q.	n.q.	n.q.	n.q.	*
Ethyl octanoate	1202	21.6±1.8	8.3 ±4.1	11.4 ±0.5	n.q.	n.q.	0.7 ±0.01	*
Isopropyl octanoate	1232	2.2±0.2	n.q.	0.04 ±0.0	n.q.	n.q.	n.q.	*
2-Methylbutyl hexanoate	1252	9.1±0.1	n.q.	5.1 ±0.3	n.q.	n.q.	n.q.	*
Ethyl 9-decenoate	1389	3.5±0.1	n.q.	n.q.	n.q.	n.q.	n.q.	*
Ethyl decanoate	1397	55.2±1	2.0 ±1.3	16.8 ±1.6	n.q.	0.1 ±0	n.q.	*
Isopropyl decanoate	1428	1.5±0.5	n.q.	n.q.	n.q.	n.q.	n.q.	*
Ethyl dodecanoate	1598	2.9±0.4	n.q.	n.q.	n.q.	n.q.	n.q.	*
Methyl palmitate	1889	8.2±1.1	n.q.	n.q.	n.q.	n.q.	n.q.	*
Total esters		552.1	294.9	125.8	9.3	3.2	0.7	

Majorero goat cheese.²³ The most abundant ester was ethyl acetate, followed by ethyl butanoate, ethyl propanoate and propyl acetate. It is notable that in Manchego cheese, ethyl esters were reported at higher levels in raw milk cheeses than in pasteurized ones.¹⁸

Terpenes

Six terpenes were identified in the cheese samples; however, higher concentrations were isolated in Kumanovo and Veles Beaten cheese (Table VI). On the contrary, terpenes were not detected in Radoviš cheeses. The most abundant terpene in the majority of the cheese samples was limonene, which is associated with citrus-like note,²⁴ and was the most abundant terpene in eleven varieties of Turkish cheese.²⁵ Three different terpene compounds were determined in Tetovo Beaten cheese, with α -pinene being the main one. α -Pinene was also identified in Kuflu²⁶ and Manchego cheeses.¹⁷ These compound originate from pasture plants and are transferred to the milk and milk products.²⁴ High levels of terpenes were detected in Resen cheese ($32.3 \mu\text{g } 100 \text{ g}^{-1}$), with limonene being the main one. Low levels of terpenes were detected in Tetova ($8.7 \mu\text{g } 100 \text{ g}^{-1}$) and Radoviš ($6.9 \mu\text{g } 100 \text{ g}^{-1}$) Beaten cheese. The majority of terpenes that were identified in the Beaten cheeses were also isolated from other types of brined cheese.²⁷ It is thought that the high level of terpenes in the cheeses could be due to the high levels of terpenes in the plants consumed by the animals. In traditional cheeses manufactured in Alpine regions, terpenes are important volatile compounds with origins in the plants that constitute the forage mixture of the pastures.²⁸ In another study, terpenes were considered to be important compounds due to their

TABLE VI. Terpenes in the Beaten cheeses, $\mu\text{g } 100 \text{ g}^{-1}$ ($n = 18$); * – significant statistical differences (Tukey's test, $P < 0.05$); n.q.: not quantified; RI – retention index

Compound	RI	Geographical origin						<i>P</i> value
		Kumanovo ($n = 3$)	Tetovo ($n = 3$)	Struga ($n = 3$)	Resen ($n = 3$)	Veles ($n = 3$)	Radoviš ($n = 3$)	
α -Pinene	934	0.13 ± 0.02	4.3 ± 1.4	n.q.	3.9 ± 0.7	2.1 ± 0.3	1.15 ± 0.17	*
β -Pinene	980	n.q.	n.q.	n.q.	1.2 ± 1.45	n.q.	n.q.	NS
β -Myrcene	990	n.q.	n.q.	n.q.	n.q.	0.2 ± 0.1	n.q.	*
<i>p</i> -Cymene	1025	n.q.	1.3 ± 1.4	n.q.	n.q.	4.2 ± 0.5	n.q.	*
l-Limonene	1033	90.5 ± 7.3	2.9 ± 1.1	n.q.	27.1 ± 3.7	56.2 ± 8.4	5.71 ± 1.80	*
γ -Terpinene	1061	0.54 ± 0.08	0.1 ± 0.02	n.q.	0.1 ± 0.1	0.4 ± 0.1	0.04 ± 0.01	*
Total terpenes		91.2	8.7	n.q.	32.3	62.9	6.9	

low odour thresholds and they were thought to originate from the plants that constituted the forage mixture of the grazing pastures.²⁸

Miscellaneous

Eleven miscellaneous compounds, including eight hydrocarbons, one phenol, one aldehyde and one sulphur compound (carbon disulfide), were detected (Table VII). Carbon disulfide was the most abundant miscellaneous compound, while toluene was isolated at various levels, as a result of contamination.²⁹

TABLE VII. Miscellaneous compounds in the Beaten cheeses, $\mu\text{g } 100 \text{ g}^{-1}$ ($n = 18$); * – significant statistical differences (Tukey's test, $P < 0.05$); n.q.: not quantified; *RI* – retention index

Compound	<i>RI</i>	Geographical origin						<i>P</i> value
		Kumanovo ($n = 3$)	Tetovo ($n = 3$)	Struga ($n = 3$)	Resen ($n = 3$)	Veles ($n = 3$)	Radoviš ($n = 3$)	
Carbon disulfide	<600	223.7 ± 18.7	17.98 ± 7	231.1 ± 16.9	513.3 ± 52.8	469.6 ± 110.4	167.5 ± 44.4	
2-Methyloctane	866	n.q.	n.q.	n.q.	n.q.	0.4 ± 0.1	n.q.	*
3,7-Dimethyl-1,6-octadiene	945	2.9 ± 0.15	4.6 ± 0.9	3.3 ± 0.6	n.q.	3.3 ± 2.9	n.q.	NS
3,7-dimethyl-2-octene	970	n.q.	4.6 ± 5.4	0.7 ± 0.1	n.q.	n.q.	n.q.	NS
2-Butenal	643	n.q.	n.q.	104.8 ± 3.9	n.q.	n.q.	n.q.	*
Toluene	763	n.q.	2.4 ± 2.8	24.8 ± 3.2	2.8 ± 0.7	2.03 ± 0.2	0.5 ± 0.08	*
<i>m</i> -Cresol	1076	4.9 ± 0.4	n.q.	0.09 ± 0	n.q.	n.q.	0.1 ± 0.01	*
Undecane	1098	n.q.	9.9 ± 3.9	n.q.	n.q.	15.6 ± 2.6	0.01 ± 0.00	*
Dodecane	1199	n.q.	n.q.	n.q.	n.q.	6.2 ± 1.3	n.q.	*
Tridecane	1301	n.q.	n.q.	n.q.	n.q.	2.1 ± 0.4	n.q.	*
Nonadecane	1846	1.5 ± 0.2	n.q.	n.q.	n.q.	n.q.	n.q.	*
Total miscellaneous		229.4	30.2	355.9	516.1	495.5	168.1	

One of the hydrocarbons, toluene, which provides nutty odour, was the most abundant hydrocarbon, already identified at high levels in Feta-type cheese.³⁰ High levels of octane were previously found in other raw milk cheeses, *e.g.*, in Spanish Manchego cheese.¹⁷ Hydrocarbons originate from fodder,¹⁸ and are also produced during the ripening as a result of lipid autoxidation.¹⁶ Volatile sulphur

compounds greatly contribute to the flavour of many cheeses¹¹ and interact with each other and with other compounds in cheese.

CONCLUSIONS

The aim of this study was to characterize the volatile profile of the cheeses that are important for the dairy sector of the FYR Macedonia. Volatile acids were the most abundant compounds isolated in the headspace analyses of Beaten cheese. These acids are of the highest importance for the aromatic profile of this type of cheese. The pattern of volatile acids formation according to their most probable origin could be associated with the different and typical characteristics in each cheese from different regions. The concentration of volatile compounds varied greatly with high standard deviations, due to the lack of standard manufacturing protocols and age-related differences. In general, the highest concentration of alcohols, esters and ketones were observed in Kumanovo, whereas the highest concentration of the miscellaneous compounds was observed in Resen Beaten cheese. This study highlights the fact that the manufacturing technique and ripening conditions of the cheeses play important roles on the formation of volatile compounds. The data presented in this article provide new information on the volatile characterization of some Beaten cheeses from different regions of the FYR Macedonian. In addition, the volatile profiles could be applied for quality control of Beaten cheeses. Complementary sensory and microbial analyses should be performed in the future to develop further the relationship between manufacturing factors and the formation of volatiles in Beaten cheese.

ИЗВОД

КАРАКТЕРИЗАЦИЈА ИСПАРЉИВИХ СУПСТАНЦИ ИЗ БИЈЕНОГ СИРА „БИЕНО СИРЕЊЕ“ МЕТОДОМ SPME/GC-MS: УТИЦАЈ ГЕОГРАФСКОГ ПОРЕКЛА

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У овом раду су окарактерисани испарљиви састојци сира који је, са економског аспекта, веома важан за БЈР Македонију. Упоредно је испитивано 18 узорака бијеног сира, из 6 различитих географских области, укључујући Куманово, Тетово, Стругу, Ресен, Велес и Радовиш. Методом екстракције у чврстој фази и гасно-масеном спектрометријом идентификован је 61 испарљив састојак. Резултати су обрађени према њиховој хемијској класификацији (17 естара, 9 кетона, 10 киселина, 8 алкохола, 6 терпена и 11 осталих једињења). Најприсутнија једињења су киселине, естри и алкохоли, а њихов састав је веома зависио од географског порекла сира. Бијени сир из Струге је имао највећи садржај карбоксилних киселина, кетона, алкохола, естара и терпена. Бијени сир из других региона је имао мали садржај испарљивих састојака, што се може сматрати последицом састава млека и начина обраде сира, који утичу на биохемијске процесе. Резултати су показали да је сир из сваке области имао различит профил

испарљивих супстанци, као и да је техника производње и стадијум зрелости сира од одлучујућег значаја за те профиле.

(Примљено 17. септембра, ревидирано 17. новембра, прихваћено 18. новембра 2013)

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