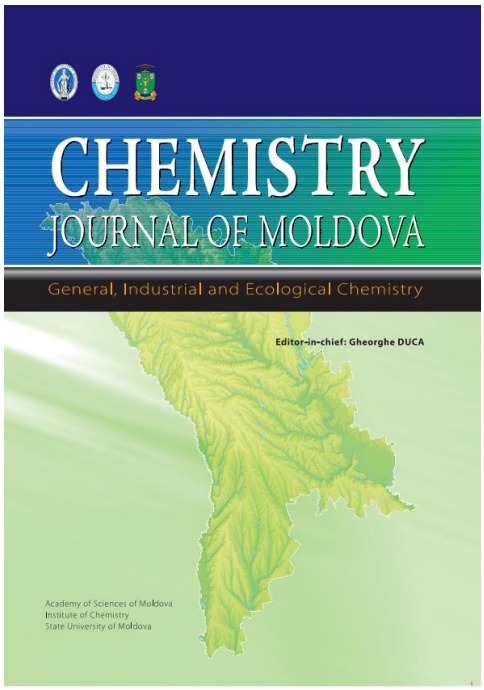


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PRELIMINARY HS-SPME-GC-MS CHARACTERIZATION OF VOLATILE COMPOUNDS IN THREE MOLDOVAN BRINED CHEESES

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Abstract. Volatile compounds in three Moldovan brined cheeses were characterized by HS-SPME-GC-MS. MBC-1 showed a high relative area of 2-butanone (25.85±1.51%), whereas MBC-2 was characterized by butanoic acid (23.89±0.17%) and a dominant unresolved acid-related signal at RT 24.66 min (46.02±2.77%). MBC-3 contained comparatively high relative areas of 3-methyl-1-butanol (24.17±6.26%), 3-methylbutanoic acid (11.21±0.60%) and 2-phenylethanol (7.44±3.04%). The observed differences represent preliminary product-specific volatile profiles and cannot be attributed independently to milk origin or processing conditions.

Keywords: HS-SPME-GC-MS, volatile compounds, goat cheese, sheep cheese, brined cheese.

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Introduction

The aroma of ripened cheeses develops through microbial and enzymatic reactions involving glycolysis, proteolysis and lipolysis, which generate carboxylic acids, alcohols, aldehydes, ketones and esters. The formation and relative abundance of these compounds are influenced by salt concentration, pH, microbiota, ripening conditions and storage [1]. Carboxylic acids frequently represent an important fraction of the volatile profile of brine-ripened cheeses, although their relative contribution varies considerably among cheese types [1].

"Brânză în saramură" is a local type of brined cheese produced in the Republic of Moldova from cow, sheep, goat or mixed milk. Salting or brining and subsequent storage or ripening under saline conditions are characteristic technological features of this cheese category and may influence its volatile composition [1,2]. Previous studies on cheese volatile profiles, including Pecorino [3] and white-brined cheeses such as Beyaz peynir [4-6], have reported carboxylic acids, alcohols, ketones and esters, with substantial variation associated with product and ripening conditions. Published studies on Moldovan brined cheese have mainly addressed physicochemical, sensory and microbiological characteristics [2].

HS-SPME-GC-MS is widely used for solvent-free characterization of cheese volatile compounds and has been applied to cheeses differing in milk source, ripening stage and processing conditions [4-7]. However, evaluation of the independent effects of these factors requires an appropriately controlled and replicated experimental design. In the present preliminary study, the three cheeses differed simultaneously in milk origin and manufacturing characteristics. Therefore, the objective was to characterize and descriptively compare the volatile profiles of three Moldovan brined cheeses rather than to determine the independent effects of milk origin or processing conditions.

Experimental

Cheese samples

Three Moldovan brined cheeses were examined and designated MBC-1, MBC-2 and MBC-3. MBC-1 was a commercially produced cheese made from pasteurized goat milk and labelled as "Brânză în saramură", with 40% fat in dry matter. It was obtained without accompanying brine. MBC-2 and MBC-3 were artisanal cheeses purchased from local market vendors. According to information provided by the vendors, MBC-2 was produced from sheep milk, whereas MBC-3 was made from a mixture of cow and sheep milk. Both artisanal cheeses were purchased in brine and transported to the laboratory in the same brine. Detailed information on their manufacturing procedures, ripening duration and brine

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composition was not available. All samples were transported under refrigeration and stored at 4°C until analysis. One product sample of each cheese was examined. Three separate HS-SPME-GC-MS analyses were performed on each sample and represented analytical rather than independent production replicates.

HS-SPME-GC-MS analysis

Volatile compounds were extracted using the cheese HS-SPME procedure described by Šaková, N. *et al.* [8]. A 5.0 g portion was placed in a 40 mL headspace vial and maintained at 50°C for 30 min while a 2 cm divinylbenzene/carboxen/polydimethylsiloxane fibre (DVB/CAR/PDMS, 50/30 μm) was exposed to the headspace. The fibre was conditioned at 270°C for 1 h before use and thermally desorbed in the GC injector at 250°C.

GC-MS measurements were carried out on an Agilent 6890 gas chromatograph coupled to an Agilent 5973N mass-selective detector. Volatile compounds were separated on an HP-INNOWax polyethylene glycol capillary column (30 m × 0.25 mm × 0.50 μm). The oven temperature was initially maintained at 50°C for 1 min, then increased to 240°C at 5°C min^{-1} and kept at the final temperature for 3 min. Helium served as the carrier gas at a constant flow rate of 1.5 mL min^{-1}. Samples were introduced in splitless mode at 250°C, and the purge valve was opened after 1 min. The transfer line and ion source were set at 230°C, while the quadrupole temperature was 150°C. Mass spectra were recorded under electron ionization at approximately 70 eV in full-scan mode over the range m/z 29-300. All measurements were performed at the Department of Chemistry and Food Analysis, Food Research Institute, National Agricultural and Food Centre, Bratislava, Slovakia.

Tentative identification and data processing

The nine chromatograms were integrated in MSD ChemStation using a single set of processing parameters. Mass spectra recorded at the apex of each peak were searched against the NIST14 and NIST17 libraries. Peak correspondence among analytical runs was established from retention-time agreement together with the predominant library match. Because neither linear retention indices nor authentic reference standards were available, all identifications were considered tentative. Signals attributed to siloxanes, phthalates or other likely analytical contaminants were omitted from the comparison.

Relative peak areas were calculated by internal normalization. A peak was included in the comparative table when it was aligned in at least two analytical runs and reached a mean relative area of 0.5% or more in at least one cheese sample. Peaks below this threshold were retained only when the spectral assignment was reproducible or when they contributed meaningfully to interpretation of the volatile profile. Where aligned peaks produced conflicting library matches, the signal was reported as uncertain rather than assigned unambiguously to a single compound.

The relative proportion of each volatile compound was calculated by internal normalization according to Eq.(1).

$$A_i(\%) = \frac{S_i}{\sum_{j-1}^{n} S_j} \cdot 100 \qquad (1)$$

where, A_i is the relative peak area of compound i, expressed as a percentage;
S_i is the integrated area of the corresponding chromatographic peak;
$\sum_{j=1}^{n} S_j$ is the sum of the areas of all integrated peaks in the chromatogram.

Results are presented as mean ± standard deviation. Inferential statistics were not applied because each cheese type was represented by one product sample. The representative full TIC chromatograms provided in the Supplementary Information (Figures S1-S3) were extracted directly from the original DATA.MS files.

Results and discussion

HS-SPME-GC-MS analysis revealed distinct volatile profiles among the three Moldovan brined cheeses. The major reproducibly detected compounds and their mean relative peak areas are summarized in Table 1, while representative full TIC chromatograms are provided in the Supplementary Information (Figures S1-S3). Because each cheese was represented by a single product sample and the products differed in several characteristics, the observed differences are interpreted as product-specific and cannot be attributed independently to milk origin or manufacturing conditions.

Ketones. Among the ketones, 2-butanone was the only compound retained in the comparative dataset. Its relative peak area differed markedly among the three cheeses, reaching 25.85±1.51% in MBC-1, compared with 2.08±0.46% in MBC-2 and 0.78±0.03% in MBC-3. Thus, 2-butanone was one of the most prominent signals in the volatile profile of MBC-1. Ketones, including 2-butanone, have also been reported in goat cheese and in white-brined cheeses [5,9]. Nevertheless, the high proportion observed

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here cannot be attributed specifically to goat milk or to pasteurization, since these factors were not examined independently.

Alcohols. The alcohol fraction showed a different distribution. Ethanol occurred in all three samples, with the highest relative area in MBC-1 (13.10±0.63%), while lower values were obtained for MBC-2 (2.23±0.86%) and MBC-3 (3.57±2.92%). In contrast, MBC-3 contained a prominent signal tentatively assigned to 3-methyl-1-butanol, accounting for 24.17±6.26% of the integrated area. The same compound represented 4.71±0.96% in MBC-1 and only 0.73±0.18% in MBC-2, where its identification was less certain. MBC-3 also contained substantially more 2-phenylethanol (7.44±3.04%) than MBC-1 (0.29±0.07%), whereas only a trace signal was observed in MBC-2. Branched-chain and aromatic alcohols such as 3-methyl-1-butanol and 2-phenylethanol can arise from amino-acid conversion during cheese ripening [1], and related compounds have been reported in both ewe-milk cheeses and white-brined cheeses [5,6,10,11]. In the present study, however, amino-acid metabolism was not measured directly; the observed pattern therefore describes the composition of MBC-3 rather than demonstrating a particular biochemical pathway.

Esters. The ester fraction was quantitatively smaller but was represented in all three cheeses. Ethyl butanoate showed relatively similar mean areas, ranging from 0.95±0.03% in MBC-1 to 1.72±0.67% in MBC-2 and 1.06±0.87% in MBC-3. A clearer difference was observed for ethyl hexanoate, which reached 5.32±1.12% in MBC-2 but remained below 1% in MBC-1 and MBC-3. Esters are regularly reported among the volatile constituents of ripened and white-brined cheeses [5,6]. Their formation may involve reactions between alcohols and carboxylic acids during ripening [1]. In this dataset, the relatively high ethyl hexanoate signal adds to the distinctive volatile pattern of MBC-2, although no conclusion can be drawn about the specific technological factor responsible for it.

Carboxylic acids. Carboxylic acids constituted an important part of the detected volatile fraction, particularly in MBC-2. Butanoic acid accounted for 23.89±0.17% of the integrated area in this sample, approximately twice the value found in MBC-1 (10.79±1.19%) and considerably more than in MBC-3 (3.00±1.46%).

Table 1

Tentatively identified volatile compounds and an unresolved chromatographic signal in three Moldovan brined cheeses analysed by HS-SPME-GC-MS.

Tentatively identified compound	*RT (min)*	*MBC-1 (area %)*	*MBC-2 (area %)*	*MBC-3 (area %)*
Ketones				
2-Butanone	2.86-2.88	25.85±1.51	2.08±0.46	0.78±0.03
Alcohols				
Ethanol	3.21-3.22	13.10±0.63	2.23±0.86	3.57±2.92
3-Methyl-1-butanol[a]	8.77-8.79	4.71±0.96	0.73±0.18	24.17±6.26
2-Phenylethanol	26.06-26.08	0.29±0.07	tr.	7.44±3.04
Esters				
Ethyl butanoate	4.91-4.92	0.95±0.03	1.72±0.67	1.06±0.87
Ethyl hexanoate	9.51-9.52	0.46±0.10	5.32±1.12	0.60±0.18
Carboxylic acids				
Acetic acid	15.44-15.49	2.45±0.63	1.24±0.14	n.d.
2-Methylpropanoic acid	18.34-18.35	n.a.	n.d.	4.50±0.46
Butanoic acid	19.68-19.80	10.79±1.19	23.89±0.17	3.00±1.46
3-Methylbutanoic acid[b]	20.75-20.78	0.37±0.06	n.a.	11.21±0.60
Octanoic acid	29.06-29.07	6.00±3.00	6.53±0.86	2.65±1.84
Decanoic acid	33.04-33.06	0.90±0.73	1.53±0.48	0.64±0.36
Unresolved signal				
Peak tentatively assigned to hexanoic acid[c]	24.66-24.69	18.42±2.68	46.02±2.77	7.49±6.43

Values are mean relative peak area ± standard deviation of three analytical runs processed using the same integration method. n.d., no aligned peak; n.a., aligned signal present but identity not reliably assigned; tr., trace (<0.10%). [a] The RT 8.77-8.79 min assignment was consistent in MBC-1, supported in two MBC-3 runs and uncertain in MBC-2. [b] The RT 20.75-20.78 min assignment was supported in MBC-1 and in two MBC-3 runs. [c] Hexanoic acid was the consistent leading library match for this signal in MBC-1, whereas alternative acid-related matches were obtained for the corresponding signal in MBC-2 and MBC-3; therefore, its exact identity remains unresolved.

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Acetic acid was detected in MBC-1 and MBC-2 at 2.45±0.63% and 1.24±0.14%, respectively, but no aligned signal was obtained for MBC-3. By contrast, 2-methylpropanoic acid was recorded only in MBC-3 (4.50±0.46%), while 3-methylbutanoic acid was particularly prominent in this sample, reaching 11.21±0.60%. The corresponding signal was minor in MBC-1 and could not be reliably assigned in MBC-2.

Medium-chain acids occurred at lower relative levels. Octanoic acid was present in all three cheeses, with mean areas of 6.00±3.00%, 6.53±0.86% and 2.65±1.84% in MBC-1, MBC-2 and MBC-3, respectively. Decanoic acid remained below 2% in every sample. Short- and medium-chain fatty acids are well documented components of the volatile fraction of ripened sheep- and goat-milk cheeses [9-11], and carboxylic acids are frequently important in brine-ripened cheeses [1,5,6]. Their presence is compatible with lipid-derived reactions occurring during cheese manufacture and ripening, but the relative peak areas reported here do not constitute direct measurements of lipolytic activity.

The comparatively high proportion of 3-methylbutanoic acid in MBC-3 is noteworthy because branched-chain acids may also originate from amino-acid degradation [1]. At the same time, compounds more specifically associated with the characteristic flavour of sheep and goat dairy products, such as 4-methyloctanoic and 4-ethyloctanoic acids, were not conclusively identified in the present chromatograms. These minor branched-chain fatty acids have been discussed as important contributors to small-ruminant dairy flavour [12-14]. Their absence from the present list therefore cautions against interpreting the detected acids as markers of a particular milk species.

Unresolved signal.

The most abundant individual chromatographic signal in MBC-2 occurred at RT 24.66-24.69 min and represented 46.02±2.77% of the integrated peak area. The corresponding signal accounted for 18.42±2.68% in MBC-1 and 7.49±6.43% in MBC-3. Hexanoic acid was the consistent leading library match for MBC-1. For MBC-2 and MBC-3, however, alternative acid-related matches were obtained in individual analytical runs. The peak is therefore retained as an unresolved signal rather than being treated quantitatively as confirmed hexanoic acid. This distinction is particularly important for MBC-2: the 46.02% value should not be added to the confirmed butanoic-acid fraction or interpreted as a measured concentration of free fatty acids.

Overall comparison.

Taken together, the three cheeses showed clearly different headspace patterns. MBC-1 was characterized by a prominent 2-butanone signal, whereas MBC-2 contained more butanoic acid, ethyl hexanoate and the dominant unresolved acid-related signal. MBC-3 differed from both through the comparatively large contributions of 3-methyl-1-butanol, 3-methylbutanoic acid and 2-phenylethanol. Several of these compounds, including butanoic acid, 3-methyl-1-butanol and 2-phenylethanol, have also been reported in Slovak bryndza [8]. Comparable volatile classes and individual compounds have been described in white-brined cheeses and Feta, including carboxylic acids, alcohols, ketones and esters [5,6,15-17]. In particular, butanoic, hexanoic, octanoic and decanoic acids, ethanol, 3-methyl-1-butanol, 2-butanone and several ethyl esters have been reported in Feta cheese [16]. These similarities place the Moldovan samples within the broader chemical context of ripened brined cheeses, but they do not imply equivalence between products.

Because each cheese type was represented by a single product and the products differed simultaneously in milk source and manufacturing history, the observed variation must be interpreted as product-specific. In particular, the present data do not separate the effects of milk species, pasteurization, microbiota, brine composition or ripening duration.

Compared with published profiles of other brined cheeses, the Moldovan samples did not contain a unique chemical class of volatile compounds. Their distinctive feature was instead the markedly different relative distribution of compounds commonly reported in ripened brined cheeses. MBC-1 was characterized by a prominent 2-butanone signal, MBC-2 by a strong acid-related profile, whereas MBC-3 contained comparatively high proportions of branched-chain and aromatic alcohols and acids. Thus, the regional interest of these cheeses appears to lie in their product-specific combinations of common cheese volatiles rather than in the presence of a single Moldova-specific marker.

Conclusions

This preliminary HS-SPME-GC-MS study revealed distinct volatile profiles in three Moldovan brined cheeses. MBC-1 was characterized by a prominent 2-butanone signal, whereas MBC-2 contained a comparatively high proportion of butanoic acid together with a dominant unresolved acid-related signal at

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RT 24.66-24.69 min. MBC-3 showed comparatively high relative areas of 3-methyl-1-butanol, 3-methylbutanoic acid and 2-phenylethanol. These differences describe the individual products examined and cannot be attributed independently to milk origin, pasteurization or specific manufacturing conditions. Because compound identification was tentative and based on library matching and relative peak areas, the results do not provide direct evidence of the underlying biochemical pathways. Further studies should include independent production batches, documented ripening and brining conditions, retention indices and confirmation of key compounds using authentic standards.

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