

DETERMINATION OF VOLATILE ORGANIC COMPOUNDS IN SLOVAK BRYNDZA CHEESE BY THE ELECTRONIC NOSE AND THE HEADSPACE SOLID-PHASE MICROEXTRACTION GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT

The aim of the present study was to describe volatile organic compounds of the traditional Slovak bryndza cheese determined by using an electronic nose (e-nose) and a gas chromatography mass spectrometry (GCMS) with head-space solid phase microextraction (HS-SPME). For the first time, e-nose based on the gas chromatography principle with a flame ionization detector was described to identify and quantify aroma active compounds of bryndza cheese from Slovakia. The e-nose detects aroma compounds of very small concentrations in real-time of a few minutes and identifies them by comparing Kovats' retention indices with the NIST library. Bryndza cheese produced from unpasteurized ewe's milk and from a mixture of raw ewe's and pasteurized cow's types of milk were collected from 2 different Slovak farms beginning in May through to September 2019. The flavour and aroma of bryndza cheese are apparently composed of compounds contained in milk and the products of fermentation of the substrate by bacteria and fungi. Regarding volatile organic compounds, 25 compounds were detected and identified by an electronic nose with a discriminant >0.900 with ethyl acetate, isopentyl acetate, 2-butanone, acetic acid, butanoic acid, and butane-2,3-dione confirmed by gas chromatography. We confirm the suitability of the electronic nose to be used for monitoring of bryndza cheese quality.

Keywords: Aroma active compounds; Ewe's cheese; Slovakia; Electronic nose

INTRODUCTION

One of the traditional ewe's milk production is bryndza cheese or Oštiepok cheese (Zajác et al., 2019; Šnirc et al., 2019). Slovak bryndza cheese is natural, white, spreadable cheese, manufactured according to the traditional method. It is recognized in the European Union by Protected Geographic Indication (PGI) status as cheese produced in specified mountainous regions of Slovakia (Commission Regulation (EC) No 676/2008; Šaková et al., 2015; Zelenáková et al., 2016) where unpasteurized ewe's milk is processed to obtain the Slovak bryndza cheese. The mountainous regions of Slovakia differ in altitude, climate, geological, and vegetation profiles and there exists some scientific evidence about the variability of bryndza which is lacking in connection to common characteristics of this Slovak cheese (Šaková et al., 2015). Ewe's cheese represents a matrix with a specific composition which reflected ewe's milk and also by different autochthonous lactic acid bacteria (LAB) produce typical aroma profile of ewe's lump cheese, barreled ewe's cheese as well as bryndza cheese (Šaková et al., 2015; Sádecká et al., 2016). Kačániová et al. (2019) identified

870 isolates from coliforms, enterococci, lactic acid bacteria, and yeasts in Slovak bryndza cheese by MALDI-TOF MS profiling. *Hafnia alvei*, *Klebsiella oxytoca*, *Lactococcus lactis*, and *Lactobacillus paracasei* were the most frequently identified species of bacteria. LAB group was represented by *Lactobacillus*, *Lactococcus*, and *Pediococcus*.

Several volatile organic compounds (VOC) of cheese, including raw milk-based ewe's cheese, are formed by proteolysis and by the subsequent transformation of amino acids (Ozturkoglu-Budak et al., 2016) to α -keto acids (Čaplová et al., 2018). Two different major pathways of amino acid degradation have been identified in *Lactococcus lactis* (Yvon and Rijnen, 2001). The first pathway is initiated by an elimination reaction of methionine catalyzed by amino acid lyases and leads to major sulphur aroma compounds (Dias and Weimer, 1998a; Dias and Weimer, 1998b). The second pathway is initiated by a transamination reaction catalyzed by aminotransferases, and has been observed especially for aromatic amino acids, branched chain amino acids, and methionine (Rijnen et al., 1999; Bourdat-Deschamps et

al., 2004). The resulting α -ketoacids are then degraded to aldehydes, alcohols, carboxylic acids, esters, methanethiol, and other sulphur compounds. Most of these compounds are produced by enzymatic degradation but a few ones result from chemical degradation in particular oxidation (Nierop-Groot and de Bont, 1998, Nierop-Groot and de Bont, 1999). VOC are usually analyzed by gas chromatography after the extraction or pre-concentration of the volatile fraction. The most exhaustive methods for this purpose are high vacuum distillation (HVT), solvent-assisted flavour evaporation (SAFE), or solid phase microextraction (SPME) (Sádecká et al., 2014) combined with headspace. Sádecká et al. (2014) used SPME with gas chromatography-olfactometry (GC-O) for the determination of volatile odorants in May bryndza cheese. Depending on the degree of cheese maturation, from a GC-O point of view, 25 olfactometric responses from groups of alcohols, aldehydes, esters, ketones, fatty acids, and hydrocarbons were recorded.

The aim of this study was to obtain, for the first time, parallel information of principal volatile organic compounds in bryndza cheese determined by an electronic nose and a gas chromatography mass spectrometry with head-space solid phase microextraction sample pretreatment and to confirm the possibility of the use of e-nose for monitoring bryndza cheese quality.

Scientific hypothesis

Hypothesis 1: The impact of the season on bryndza cheese aroma profile determined by e-nose.

Hypothesis 2: The impact of the season on bryndza cheese aroma profile determined by HS-SPME-GC-MS.

Hypothesis 3: The application of the method to monitor bryndza cheese evaluation.

MATERIAL AND METHODOLOGY

Bryndza cheese samples

Samples of bryndza cheese were provided by 2 different producers. The first sample was produced from unpasteurized ewe's milk by farm dairy. The second one was produced from a mixture of raw ewe's (min. 50%) and pasteurized cow's milk by industrial dairy. Samples were collected from May to September 2019. All samples (10) were placed in sterile sample containers and transported to the laboratory on ice. Fresh samples were analyzed by head-space solid phase microextraction gas chromatography mass spectrometry (HS-SPME GC-MS) and electronic nose (e-nose) within one day after the delivery.

E-nose analysis

The electronic nose method (e-nose Heracles II, Alpha M.O.S., Toulouse, France) previously described by Štefániková et al. (2019) was used for sample analysis. For each analysis, 2.5 g of sample was incubated statically in a 20 mL vial in a thermostat block at 50 °C for 15 min (Autosampler, Alpha M.O.S.) and 5 mL volume of the headspace gaseous compounds was withdrawn using a headspace autosampler syringe and dispensed into the e-nose injector. The identification of compounds was performed by matching the measured peaks with Kovats' retention indices with the NIST® library (The National

Institute of Standards and Technology library) (>50%) by software Alpha Soft V14 (Alpha M.O.S.).

HP-SPME-GC-MS analysis

The head-space solid phase microextraction method was used for a sample extraction according to Sádecká et al. (2014) in a modified version. For each analysis, 2.5 g of sample was incubated statically in a 20 mL vial in a thermostat block at 50 °C for 30 min (CombiPal Autosampler 120, CTC Analytics AG, Zwingen, Switzerland), with an SPME fibre (1 cm; DVB/CAR/PDMS) (Supelco, Bellefonte, PA, USA) placed in the CombiPal.

Semi-quantitative composition of samples was determined by gas chromatography coupled with mass spectrometry (GC-MS) using an Agilent 7890B oven coupled with Agilent 5977A mass detector (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with column DB-WAXms (30 m × 0.32 mm × 0.25 µm; Agilent Technologies) operating with a temperature program and MS conditions according to Sádecká et al. (2014).

The identification of compounds was carried out by comparison of mass spectra (over 80% match) with the NIST® 2017 library and retention times of reference standards (ethyl acetate, hexanoic acid, and isopentanol). The semi-quantitative content of determining compounds was calculated by dividing the individual peak area by total area of all peaks. Peaks under 1 % were not counted.

Statistical analysis

Compounds identified by e-nose with a discriminant >0.900 were selected, based on which the semi-qualitative evaluation was performed and PC analysis (Principal Component Analysis) was made by Alpha Soft V14 (Alpha M.O.S.) software. Descriptors were analyzed using single factor analysis of variance and significance was at $p < 0.05$.

The STATGRAPHICS Centurion (© StatPoint Technologies, Inc., USA) and GraphPad Prism 6.01 (GraphPad Software Incorporated, San Diego, California, USA) were used for statistical GC-MS analysis. The ANOVA method complemented by the Test of Tukey's Multiple Comparison Test with a value of $p < 0.05$ was applied.

RESULTS AND DISCUSSION

In this study, the aromatic profiles of ten bryndza cheese samples by e-nose and HS-SPME GC-MS were evaluated. Bryndza cheese samples were collected from the Slovak dairies. Bryndza cheese is a soft spreadable cheese, made from unpasteurized ewe's milk or a mixture of ewe's and cow's milk. Kačániová et al. (2019) previously described the microbiota studies of ewe's bryndza cheese from the same mountainous regions of Slovakia. LAB, enterococci, and yeasts *Galactomyces candidus* play a key role in flavor development during cheese ripening (Kačániová et al., 2020; Pangallo et al., 2014; Sádecká et al., 2019). The VOC's are generated by the enzymatic degradation of amino acids in cheese, especially in cheese containing the only LAB.

Table 1 Determination and identification of volatile organic compounds with a discriminant >0.900 in bryndza cheese samples by e-nose.

Compounds		Compounds	
1	ethyl acetate	14	benzaldehyde
2	isopropyl acetate	15	acetaldehyde
3	isopentyl acetate	16	furfural
4	butyl acetate	17	2,3-butandione
5	ethyl propanoate	18	2-pentanone
6	ethyl butyrate	19	2-butanone
7	acetic acid	20	benzyl alcohol
8	butanoic acid	21	n-butanol
9	propanoic acid	22	2-propanol
10	propanal	23	1-hexanol
11	butanal	24	2-methyl propanol
12	3-methyl butanal	25	α-pinene
13	2-methyl propanal		

The amino acid transamination is catalyzed by lactococci aminotransferases and it is the first step in the degradation of aromatic and branches-chain amino acids which are precursors of aroma compounds (Yvon et al., 1997; Tanous et al., 2005). The resulting α-ketoacids are then degraded to aldehydes, alcohols, fatty acids, esters, methanethiol, and other sulphur compounds (Savijoki, Ingmer and Varmanen, 2006).

The identification of the compounds determined by e-nose was performed by matching the measured peaks with Kovats retention indices with the NIST library. Aroma compounds identified in bryndza cheeses by e-nose are shown in Table 1. Total, 25 compounds with a discriminant >0.900 from class alcohols, esters, fatty acids, terpenes ketones, and aldehydes were identified. Ten compounds – 3-methyl butanal, 2-methyl-1-propanol, 2-butanone, 2-pentanone, ethyl acetate, isopentyl acetate, ethyl butyrate, acetic acid, butanoic acid, and butane-2,3-dione were identified in this study by e-nose and by gas chromatography-olfactometry previously described in

bryndza cheese from Slovakia by Sádecká et al. (2014, 2016, 2019) and Šaková et al. (2015).

Identified compounds by e-nose with a discriminant >0.900 were selected, based on which the semi-qualitative evaluation was performed by the principal component analysis (PCA). Figure 1 displays result processed by the PCA technique of the aroma profile of bryndza cheese samples. The first dimension (PC1 86.927%) allows the separation of July2 and August2 (positive score) from the other samples of cheese (negative score). Among samples May1, July2 and September2 (negative score of the second dimension PC2; 9.402%) and other samples of cheese (positive score PC2) statistically significant differences (p <0.05) were evident in their aroma profiles.

We confirmed differences in aroma profiles between May bryndza and summer or winter bryndza produced from ewe’s milk by the first dairy. On the contrary, more aroma profile differences were in bryndza cheese produced from the mixture of ewe’s and cow’s milk by the second dairy.

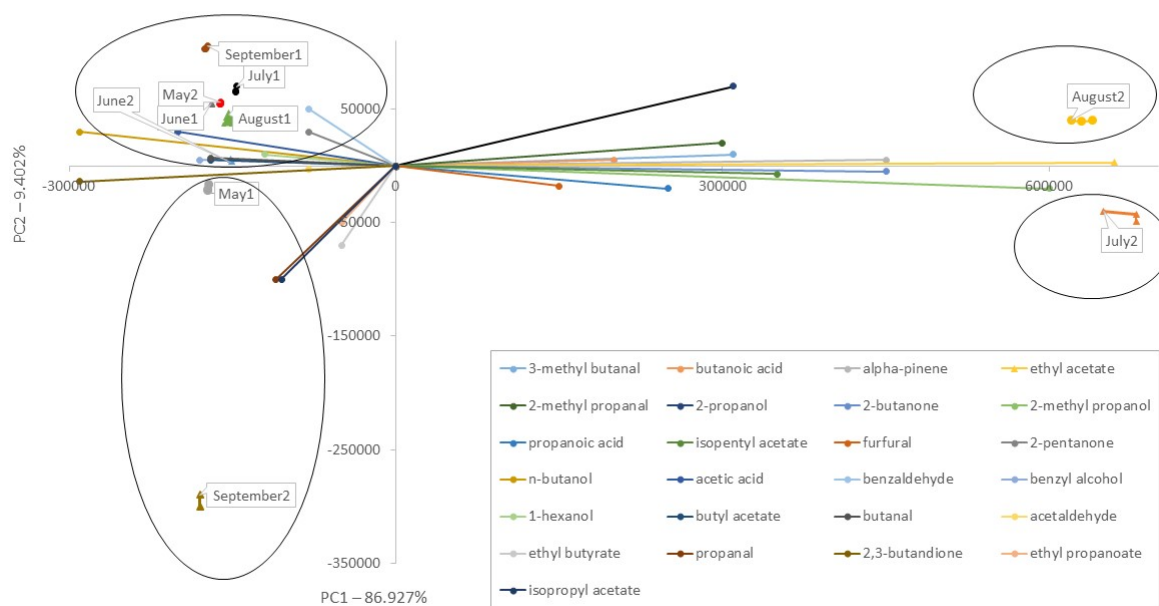


Figure 1 PCA analysis of the aromatic profile of the bryndza cheeses acquired by e-nose.

The samples collected in May and June showed no significant differences in aroma profiles when compared to each other but the aroma profiles were significantly different in comparison with July, August, and September samples and at the same time, the samples collected from July, August, and September showed statistically different ($p < 0.05$) aroma profiles compared to each other.

On the contrary, the position of May2, June2, June1 – September1 samples on the negative score of axis 1 and a positive score of axis 2 could be explained by its higher proportions of acetic acid, benzyl alcohol, butyl acetate, butanal, benzaldehyde, n-butanol, 2-pentanone, and hexanol. The August2 sample is positioned at the opposite on axis 1 due to its higher proportions in butanoic acid, 2-propanol, 2-methyl propanal, 3-methyl butanal, α -pinene, and ethyl acetate. The sample of July2, positioned on the positive score of axis 1 and the negative score of axis 2, contains higher proportions of isopentyl acetate, 2-butanone, 2-methyl propanol, furfural, and propanoic acid and the position of May1 and September2 samples on the

negative scores of axes 1 and 2 could be explained by their higher proportions of ethyl butyrate, ethyl propanoate, propanal, 2,3-butanedione, and acetaldehyde.

Semi-quantitative content of identified volatile organic compounds in bryndza cheese samples determined by GC-MS is shown in Table 2. In total, 6 higher alcohols, 3 esters, 5 fatty acids, 3 ketones, and methoxy-phenyl-oxime were determined in the samples. Findings showed that principal odorants detected in all samples were acetoin (3.88% – 27.1%), acetic acid (1.77% – 18.7%), dimethyl-silanediol (1.07% – 6.93%), methoxy-phenyl-oxime (1.78% – 7.01%), and isopentanol (1.72% – 7.44%).

The bryndza cheese from the first dairy contains about 15% of acetoin collected from May and June, and, on the contrary, the next months its content decreased (10.6%, 7.04%, and 10.5% respectively). The content of acetoin in samples from the second dairy was more variable, its amount was decreased (3.88%) in samples collected from July production and increased in samples collected from August production (27.1%).

Table 2 Semi-quantitative content (area %*) of identified volatile organic compounds in bryndza cheeses produced from ewe's milk (1 – first dairy) and from mixture with cow's milk (2 – second dairy) collected from May – September determined by GC-MS.

Compounds	Bryndza cheese samples									
	May1	May2	June1	June2	July1	July2	August1	August2	September1	September2
Area %										
1 ethyl acetate	1.94	2.62		4.65			10.27			
2 2-phenethyl acetate	0.95	1.08					16.6	0.62		
3 isopentyl acetate							1.76			
4 2-butanone ^b						16.2		16.8		
5 2,3-butanedione (diacetyl)		1.55						2.35	3.55	
6 3-hydroxy-2-butanone (acetoin)	15.2	16.8	15.7	18.5	10.6	3.88	7.04	27.1	10.5	9.73
7 acetic acid ^a	9.00	9.13	11.0	8.55	9.69	13.3	1.77	6.92	18.0	18.7
8 butanoic acid ^a	1.89	1.84	2.21	2.03	1.91	2.11		1.22		
9 pentanoic acid ^b	0.73							1.28		
10 hexanoic acid ^a	3.92	4.07	3.19	2.00	4.10	3.10		2.22		
11 octanoic acid ^a	1.51	1.37			2.42					
12 2-butanol						12.4				
13 2,3-butanediol ^a	3.83	3.67			2.41		1.54		5.42	4.21
14 2,7-dimethyl-4,5-octanediol ^a	1.01	1.06								
15 dimethyl-silanediol ^a	3.99	3.98	2.17	2.3	3.03	2.48	1.07	2.34	6.93	6.16
16 3-methyl-1-butanol (isopentanol)	5.03	4.69	2.06	2.82	3.41	3.21	7.44		1.72	2.41
17 2-phenyl ethanol	1.05	1.32					12.40	0.61		
18 methoxy-phenyl-oxime ^a	5.13	4.54	7.1	7.01	5.48	5.03	1.87	3.29	2.44	1.78

Note: *listed are the components that represented min. 1% in at least one bryndza cheese sample. Letters in superscript indicates statistically significant difference: a – among samples depending on month of production, b – among samples depending on kind of milk.

The content of acetic acid was in a range of 8.55% – 13.3% in samples produced in May–July, it was decreased in August (1.77% and 6.92%, respectively) and increased in September (18.0% and 18.7%, respectively). While acetoin and acetic acid were the most representative compounds in samples produced in May and June, samples produced in July and August had different profiles. While the samples from the first dairy had a higher content of acetoin and acetic acid (July) and 2-phenethyl acetate and 2-phenyl ethanol (August), the samples from the second dairy had a higher content of 2-butanone and acetic acid (July) and 2-butanone and acetoin (August). Bryndza cheese produced in September had a weaker aroma, but acetic acid and acetoin were identified in the higher amount than in the May and June samples. Statistical analysis by the Test of Tukey's Multiple Comparison Test confirmed that the amount of eight aromatic compounds (Table 2) was influenced by the month of production. On the other hand, only two aroma substances were notably influenced by the kind of milk.

The compounds identified by the electronic nose with a discriminant >0.900 with ethyl acetate, isopentyl acetate, 2-butanone, acetic and butanoic acids, and butane-2,3-dione were confirmed by GC-MS. Several of the identified compounds (ethyl acetate, 2-phenylethyl acetate, ethyl propanoate, 2-methyl-propanol, 2-phenyl ethanol, 2,3-butanediol, 3-hydroxy-2-butanone (acetoin), 3-methyl butanol, 2,3-butanedione, acetic, butanoic, pentanoic, and octanoic acids) are known to be components of different foreign ewe's cheese such as the Oscypek, Canestrato Pugliese, Fiore Sardo, Torta del Casar, Terrincho, Roncal, Manchego, and Pecorino Romano (Barron et al., 2005; Massouras, Pappa and Mallatou, 2006; Majcher et al., 2011; Sádecká et al., 2014; Delgado-Martínez et al., 2019).

Other identified compounds, benzaldehyde (Bourdat-Deschamps et al., 2004), 2-pentanone (Gallegos et al., 2017), hexanal (Gómez-Torres et al., 2016), 2,7-dimethyl-4,5-octanediol (Nájera-Domínguez et al., 2015) were previously described as secondary metabolites by LAB. Passerini et al. (2013) confirmed that strains of *L. lactis* with the *citP* gene and the *citM-G* cluster produced a larger amount of aroma-active compounds than the strains without this genetic information. Bozoudi et al. (2018) and Iussig et al. (2015) reported the quality differences of milk and dairy products from different grazing areas. The terpenoid composition (limonene, myrcene, carvone) of milk and cheese are directly transferred from ingested botanical species and free fatty acids (from acetic to dodecanoic acid) can also be effective to trace animal management and feeding systems (Moran et al., 2019). The free fatty acids are also precursors of methyl ketones, alcohols, lactones, and esters, so they may play an important role in the global aroma development of cheese (Delgado-Martínez et al., 2019). Fatty acids were the most abundant VOCs in the barrelled ewes' types of cheese (intermediate product in the production of winter bryndza) from Slovakia (Sádecká et al., 2016) and in the raw ewe's milk cheese, Feta cheese and Torta del Casar (Bozoudi et al., 2018; Delgado et al., 2010; Delgado-Martínez et al., 2019). The identified 2-methyl propanal (Griffiths, 2010) was previously described as milk aromas. The other compounds, n-butanol, dimethyl-

silanediol, and methoxy-phenyl-oxime were identified in cheeses produced from pasteurization milk fermented with mixed cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) and with *Dregea sinensis* Hems. protease (Wang, Wang and Huang, 2017).

The e-nose technology in this study can detect the fingerprint of volatile organic compounds present in the headspace of the bryndza sample and parenica cheese previously described (Štefániková et al., 2019) by the means of a gas chromatography principle. Several studies used an e-nose method containing 10 metal-oxide semiconductors for characterization of the aroma profile of French cheese types, Danish blue cheese, or Pecorino cheese (Ghasemi-Varnamkhasti et al., 2019; Trihaas, Vognsen and Nielsen, 2005; Cevoli et al., 2011). There was used the e-nose with sensors in the above-mentioned studies, which could not determine or identify concrete volatile organic compounds and therefore there was a need to confirm the results by GC methods.

CONCLUSION

This study has proved for the first time the possibility of bryndza cheese quality evaluation using an e-nose with GC columns and FID detectors. The results were compared with gas chromatography with mass spectrometry. The e-nose method can determine the aroma profile of samples in a short time and the results may be supplemented by sensory evaluation by the assessors. The e-nose may take great advantages over GC-MS in distinguishing the integral aroma profiles, although it cannot identify the explicit volatile compounds of different samples.

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