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APPLICATION OF ELECTRONIC NOSE FOR DETERMINATION OF SLOVAK CHEESE AUTHENTICATION BASED ON AROMA PROFILE

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ABSTRACT
Electronic nose with sensors is used in many industries and for various applications such as quality control, process monitoring, shelf life evaluation, origin or authenticity assessment. The aim of this work was to investigate the electronic nose with FID detectors applicability for characterization of steamed cheese and for the assessment of steamed cheese quality decay during storage. Samples of smoked and unsmoked steamed cheese varieties from 5 Slovak enterprises concerning different regions of Slovakia were analysed. Data from aroma profiles were processed by statistical technique PCA. Compounds like acetaldehyde, 1-propanal, propanoic acid, ethyl hexanoate, furfural, butan-2-one, isovaleric acid, 1-hexanol or α-pinene were determined as significant flavours in fresh steamed cheese samples. In the current study, no significant differences in aroma profiles between fresh and stored cheese samples were confirmed. Thus, differences in main odour substances composition of steamed cheese varieties, obtained from various producers in several geographic regions of Slovakia, were minor.

Keywords: Slovak steamed cheese; aroma profile; e-nose; authentication

INTRODUCTION
Electronic nose (e-nose) is an odour detection device using a sensor array **(Delgado-Rodríguez et al., 2012)**. Many industries use e-nose for diverse applications such as quality control **(Li et al., 2017; Xu et al., 2017; Gancarz et al. 2017; Chen et al., 2018; Buratti et al., 2018)**, process monitoring, durability assessment, origin ranking and originality **(Wilson and Baietto, 2009; Śliwińska et al., 2014; Li et al., 2017)**. Another implementation of e-nose in the food industry involve dairy products classification whether in terms of flavour, variety type, geographical origin, ripening stage (in case of cheese) or its shelf life prediction **(Ampuero and Bosset, 2003)**. E-nose can be used also to monitor volatile compounds that indicate female cattle fertile period, acknowledged by the study of **Manzoli et al. (2019)**.

Working principle of e-nose Heracles II is gas chromatography, detecting aroma compounds of very small concentrations in real-time of a few minutes and identifying them by comparing Kovats retention indices with the NIST library. To obtain vital information from the analysis of samples, multivariate statistics is applied most frequently **(Buratti et al., 2018)**. The use of e-nose analysis is a rapid, easy, reliable, accurate and non-polluting method practice.

Aroma perception occurs by olfaction, when olfactory receptors placed on the nasal cavity roof are stimulated by aroma active compounds. In terms of flavour recognition during food or beverage consumption, the aroma active compounds are perceived retronasally. Different combinations of large variety of compounds including short, medium chain fatty acids, alcohols, aldehydes, ketones, esters or sulphur compounds create a characteristic smell and flavour of each and every cheese product. Multiple cheese types may consist of the same aroma active compounds, yet they differ from each other by altered amount, and therefore the percentage content, of particular component in such product **(Niimi et al., 2014)**. According to **Commission Regulation (EC) No 656/2008**, the protected geographical indication "Slovenská parenica" is an official label of Slovak parenica type steamed cheese if it meets all characteristics and requirements defined within this Regulation (referring to Council Regulation (EC) No 510/2006). Here, Slovak parenica is defined as "a steamed, lightly smoked cheese wound into two rolls 6 – 8 cm in diameter and 5 – 8 cm high, connected in a 'S'- shape having yellow to brown colour on the outside after smoking; white to buttery yellow on the inside. The rolls are bound with cheese string or chain. Prior to being rolled up, the cheese strip is 2 – 3 mm thick, 5 – 8 cm wide and 4 – 6 m long. The ingredients used are fresh raw, unprocessed ewe's milk from grazing ewes of the Wallachian, improved Wallachian, Cigaja and East Friesian breeds or a mixture of fresh raw, unprocessed ewe's milk and fresh raw, unprocessed cow's milk, containing at least 50% ewe's milk". In Slovakia, the production of parenica type cheese is very widespread, directed either as mechanized production for big dairy factories or as manual operation by small corporations.

In presented study, Slovak steamed cheese samples, produced by small and medium manufacturers from pasteurized raw cows' milk, were analysed. Being processed from cow's milk, they are not referred to as Slovenská parenica. For this work`s purposes, we will refer to the analysed samples as the steamed cheese in the following text. At the beginning of the steamed cheese production, a starter culture is added to the milk for the fermentation process and thereby to reduce the pH of the milk to the desired level. For such aim, mesophilic culture (*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*) and thermophilic culture (*Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*) are the most commonly used ones **(Onipchenko et al., 2012)**. After the starter culture inoculation, the rennet is added, and the coagulation phase of milk begins. The optimum coagulation temperature is 30 to 35 °C. The created cheese curd is then treated by cutting, stirring and heating to a higher temperature, resulting in separating the whey out and thereby reducing the water content. The raw cheese block is then pressed with gentle pressure to promote the separation of the whey and such a cheese block is then an input raw material for the steaming process. The steaming process runs in two stages. In the first steaming stage, the shredded cheese curd is dosed into a hot water tank (65 – 85 °C). Due to the high temperature, the raw cheese dough gets a stretchable and mouldable consistency. In the second steaming stage, the raw dough is stretched and kneaded. After these phases, the next step in steamed cheese production is forming the cheese into desired shapes. Finally, the steamed cheese is subjected to a salt bath and in case of smoked cheese products the salted cheese undergoes smoking process **(Muliawan and Hatzikiriakos, 2008; Zimanová et al., 2016)**.

Scientific hypothesis

The aim of this study was to determine differences between steamed cheese products and to confirm the ability to evaluate the authentication of these products using the fast method of electronic nose with FID detectors.

MATERIAL AND METHODOLOGY

Sampling

Samples of smoked (S) and unsmoked (U) steamed cheese varieties from 4 Slovak enterprises classified as medium enterprises (samples no. 1, 2, 4, 5) and one small enterprise (sample no. 3) from different regions of Slovakia were collected. Samples were processed from pasteurized cow's milk. Sampling was performed on the day of production, and analysis of fresh samples was carried out on the following day. Subsequently, analyses of samples were performed again after storing the samples for 14 days at 4 – 8 °C. Samples were stored in original packaging bags sealed under protective atmosphere conditions in refrigerator at temperature 4 – 8 °C.

Sample preparation

Steamed cheese samples were sliced into small pieces, and by 4 g weighed into clean 20 mL headspace vials closed by magnetic cap with PTFE/Sil septum. Thus, prepared samples were stirred at 50 °C for 15 minutes on a shaker included as a part of the GC headspace autosampler (Combi Pal, Alpha M.O.S.).

Determination of aroma profiles

E-nose with two FID detectors (Heracles II, Alpha M.O.S.) was used for determination of steamed cheese samples aroma profiles. From the saturated air above the sample level, 5 mL volume was withdrawn using a headspace autosampler syringe and dispensed into the e-nose injector heated to 200 °C. The analysis itself lasted for 110 seconds and the separation of aroma compounds took place in two columns with following temperature program; for the 1st column: isotherm 80 °C, for the 2nd column: initial temperature 50 °C and temperature gradient 3 $°C.s^{-1}$ to 250 °C. Hydrogen was used as the carrier gas. Identification of the compounds was performed by matching the measured peaks with Kovats´retention indices with NIST library (The National Institute of Standards and Technology library) by software Alpha Soft V14 (Alpha M.O.S.).

Statistical analysis

Compounds with a discriminant >0.990 were selected, based on which the semi-qualitative evaluation was performed and PC analysis (Principal Component Analysis) was made by Alpha Soft V 14 (Alpha M.O.S.) software. Descriptors were analysed using single factor analysis of variance and significance was at p <0.001.

RESULTS AND DISCUSSION

Thirteen specified aromatic compounds with a discriminant >0.990 were selected, based on which the semi-qualitative evaluation was performed and PCA analysis was made. Figure 1 displays results processed by PCA technique of the aroma profile of fresh steamed cheese samples. Among smoked and unsmoked fresh steamed cheese from the suppliers no. 1, no. 2 and no. 3 (negative scores), and no. 4 and no. 5 (positive scores) no statistically significant differences (p <0.001) were evident in their aroma profiles (PC1 axis 95.94%). On the contrary, samples of smoked (negative scores) and unsmoked (positive scores) fresh steamed cheese from suppliers no. 2, no. 3, no. 4 and no. 5 were differed in their aroma profiles in the PC2 axis (3.34%). It is assumed that these manufacturers use a different way for cheese smoking in terms of wood used in combustion process.

Cheese types such as Italy mountain cheese, Camembert cheese, Mozzarella cheese, Cheddar cheese etc. are also the subject of number of studies **(Carafa et al., 2019; Batty, Waite-Cusic, Meunier-Goddik, 2019; Kim et al., 2014; Velasco et al., 2019)**, which are of a different cheese type as our investigated samples are, and therefore it is not possible to comapre the results of the Slovak steamed cheese samples with the results of the studies mentioned above. The aroma profile of unripened steamed cheese produced from cows' milk is not fully investigated and reported, and therefore the results might not correlate with results of the authors of other studies.

Semi-qualitative evaluation was based on comparison of the Kovats` retention indices with the NIST library. Samples were divided into three groups, each consisting of samples with only small differences in their aroma components composition. As significant compound (discriminant >0.990) responsible for the fresh steamed cheese aroma, 1-propanal was detected in samples 1S, 1U, 3U and 2U. Differences causing compounds in aroma profiles of 2S and 3S fresh samples, compared to the other samples, were acetaldehyde, ethyl hexanoate, isovaleric acid, 1-hexanol, furfural, butan-2-one and α-pinene. **Bhandari et al. (2016)** determined the aromatic profile of ripened Italian Parmigiano Reggiano cheese from unpasteurized milk by SPME-GC-MS and detected of furfural, ethyl butanoate, ethyl hexanoate, ethyl octanoate, 2-nonanone, 2-heptanone, 3-methyl butanal, acetic, butanoic, hexanoic, octanoic and decanoic acids and benzeneacetaldehyde as the major compounds. On the contrary, carboxylic acids were the most abundant aroma compounds in the Torta del Casar cheese performing SPME-GC-MS analysis, specifically acetic, butanoic, hexanoic and octanoic acids **(Delgado-Martínez et al., 2019)**.

Smoked and unsmoked fresh steamed cheese samples from suppliers no. 4 and no. 5 differed from other samples by the presence of propanoic acid and 1-propanal in their aroma profiles composition. Identified compounds matched the NIST library with ≥50%. Therefore,

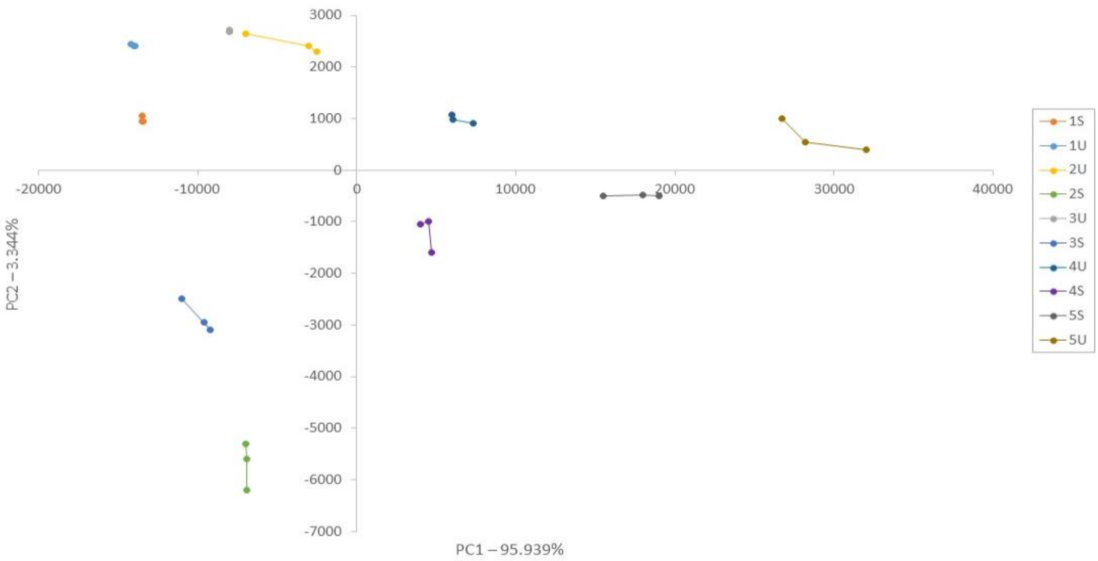

Figure 1 Projection of the samples onto the space defined by the first two principal components (PC1/PC2). Sample groups according to fresh: S (smoked steamed cheese) and U (unsmoked steamed cheese) of five Slovak producers (1 – 5).

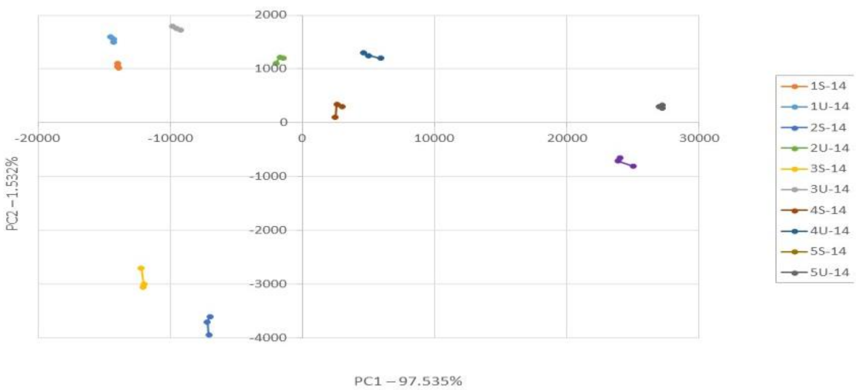

Figure 2 Projection of the samples onto the space defined by the first two principal components (PC1/PC2). Sample groups according to stored: S (smoked steamed cheese) and U (unsmoked steamed cheese) of five Slovak producers (1 – 5).

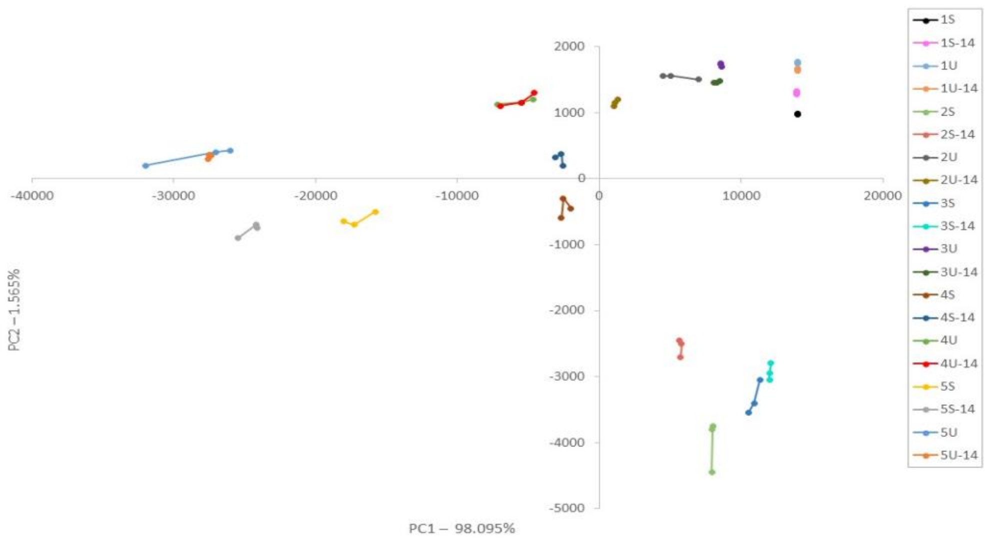

Figure 3 Projection of the samples onto the space defined by the first two principal components (PC1/PC2). Sample groups according to storage conditions: S (smoked steamed cheese) and U (unsmoked steamed cheese) of five Slovak producers (1 – 5).

verification of presented claims for comparison of aroma profiles by GC-MS or GC-FID-O analysis is highly recommended **(Sádecká et al., 2014; Šaková et al., 2015; Sádecká et al., 2016)**.

Samples were stored in the original sealed packages in refrigerator for 14 days, after which their aromatic profiles were determined the similar way as the fresh samples. The stored smoked steamed cheese samples 2S-14, 3S-14 nad 5S-14 were found in the negative scores of PC2 axis, while stored unsmoked samples from the same suppliers (2U-14, 3U-14 and 5U-14) were plotted in the positive PC2 axis 1.53%. There were no differences in the aroma profiles of the samples from the suppliers no. 1 and no.4 (1S-14 and 1U-14, 4S-14 and 4U-14) (PC2).

Evaluation was done by comparing Kovats` retention indices with NIST library (≥50%) and PCA analysis (see Figure 2). In case of stored steamed cheese samples, the number of compounds generating differences in the aromatic profile with discriminant >0.990 increased to 21 as oppose to the fresh ones. In analogy to the fresh samples, similar relationships between samples were observed, with propanal, acetaldehyde, isovaleric acid, 1-hexanol, furfural or α-pinene as predominating compounds. For smoked samples from suppliers no. 2 and no. 3 compounds such as diacetyl, 2-pentanone, 3-heptanone, 2-heptanone, ethyl hexanoate, hexanal and 2-methylpropanal. For stored samples no. 4 and no. 5, except propanoic acid and propanal, 2-methylpropanol was also a significant component of their aroma profiles, yet it was not recorded as significant in the equivalent fresh samples of the same suppliers.

For example, **Majcher et al. (2011)** studied a special type of Polish smoked (1 or 3 days) ewe cheese called Oscypek. Analysed smoked cheese consisted compounds from biochemical reactions (carboxylic acids, alcohols, aldehydes, ketones, esters, sulfur compounds), from smoking (furans/furanones, phenols) and from milk flavour (terpenes). The results of our research, analysing aroma profiles of Slovak steamed cheese by e-nose, confirm the presence of above mentioned compounds (furfural, propanoic acid, diacetyl, ethyl hexanoate, etc.) and compounds alike (2-butanone, propionaldehyde, etc.). **Thomsen et al. (2014)**, examined the aroma profile of seven commercial semi-hard cheese samples by GC-MS and their study is to some extent comparable to the aroma profile (2-propanol, 2-methyl-propanol, 3-methyl butanal, diacetyl, ethyl acetate, ethyl propanoate, ethyl butyrate, propanoic acid) of this work`s steamed cheese samples. **Guarrasi et al. (2017)** examined the effect of starter and non-starter lactic acid bacteria on the aromatic profile of Caciocavallo Palermitano cheese, traditional cheese in Western Sicily. The class of ketones represented a consistent percentage of the volatile compounds, followed by alcohols and esters. 2-butanol, butanoic and hexanoic acids and their esters, diacetyl and 3-hydroxy-2-butanone have been identified as significant compounds of volatile profiles of cheese by lactic acid bacteria. The identified α-pinene from terpenes group is usually introduced to cheese flavour as milk constituents **(Nogueira, Lubachevsky and Rankin, 2005; Majcher et al., 2011)**.

No statistically significant differences (PCA) (p <0.001) in aroma profiles of fresh and stored samples were detected when comparing samples from the same supplier, depicted in Figure 3, except smoked fresh (negative axis PC2) and stored (positive axis PC2) samples from the supplier no. 4.

CONCLUSION

This study for the first time proved possibility of steamed cheese quality evaluation in only a few minutes using e-nose. This simple and rapid method could be implemented also during sensory evaluation by tasters, allowing and granting a fast and inexpensive analysis of the controversial samples.

The results of this research have shown that odours of steamed cheese, obtained from several producers of different geographic Slovak regions, varied only a little in terms of the main representative aroma substances by means of e-nose detection. Slight differences could be caused due to the use of cows' milk instead of ewe's milk, due to the different geographical area, and also due to the presence of various non-starter and starter dairy bacteria. The 2S and 3S samples had a different aromatic composition compared to other samples, thus opening up the space for evaluation of the cheese smoking effect on the aroma profile of the smoke vapours. For confirmation of submitted conclusions, it is appropriate to measure a larger number of samples and therefore obtain extensive data of the supplied products.

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