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COMPARISON OF ORIGINAL AND ADULTERATED OSCYPEK CHEESE BASED ON VOLATILE AND SENSORY PROFILES*

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Background. This paper describes a preliminary studies aiming to compare volatile fractions of Oscypek and oscypek-like cheeses with SPME-GC/TOFMS to determine the possibility of applying for future routine investigation of adulteration of Polish PDO cheeses.
Material and methods. For sensory and volatiles analysis four different cheeses were compared: Oscypek cheese prepared according to PDO regulations and three oscypek-like cheeses: type "CM industry" – produced from pasteurised cow milk in dairy plant, type "EM-industry" – produced from pasteurised ewe milk in dairy plant and type "CM-shepherds" – produced from unpasteurised cow milk in shepherds huts. Isolation of volatiles was performed with PDMS/CAR/DVB fiber. Compounds identification was performed using gas chromatography coupled to time of flight mass spectrometry.
Results. Headspace SPME-GC/TOFMS method revealed a total of 51 compounds in Oscypek and oscypek-like cheeses representing nine chemical groups such as: free fatty acids, esters, ketones, alcohols, aldehydes, furans and furanones, phenols, sulfur compounds and terpenes. Results showed that original Oscypek, PDO labeled was represented by the largest number of volatiles identified compared to oscypek-like cheeses, which also showed a relationship with sensory analysis where Oscypek has been described as a cheese with mostly developed flavour bouquet. Additionally it could be observed that cheeses made from unpasteurised milk using traditional method of preparation in shepherds huts (Oscypek and CM-shepherds) had superior volatile profiles and enhanced aroma compared to cheeses made industrially.
Conclusions. The differences showed in volatile fraction of original Oscypek cheese and adulterated ones provide possibility of employing SPME-GC/TOFMS technique to find adulteration in PDO labelled Oscypek.

Key words: Oscypek, flavour compounds, adulteration, PDO products, gas chromatography, mass spectrometry, quality

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INTRODUCTION

Oscypek is probably the most famous among all Polish traditional cheeses. It is a smoked scalded hard cheese made from raw sheep milk by Polish shepherds living in the Tatra Mountain. The unique flavour of Oscypek described as slightly sour, piquant, salted and smoked, is conditioned by many factor such as: milk flavour of special breed of sheep use of natural microflora and traditional technology of production which includes hand processing, application of wooden tools and natural preservation methods such as brining and smoking in shepherds huts. Although Oscypek has been made in Poland for more than 200 years its standards of production have been regulated only just in February 2008 by PDO (Protected Designation of Origin) Regulatory Board. The PDO label (in Poland it is Ch.N.P. Chroniona Nazwa Pochodzenia) is designed for foods manufactured and matured in a defined geographical area possessing unique human and natural characteristic. Main stages of Oscypek preparation are presented in Figure 1 and details are included in Official Journal C180 02.08.2006 ( ).

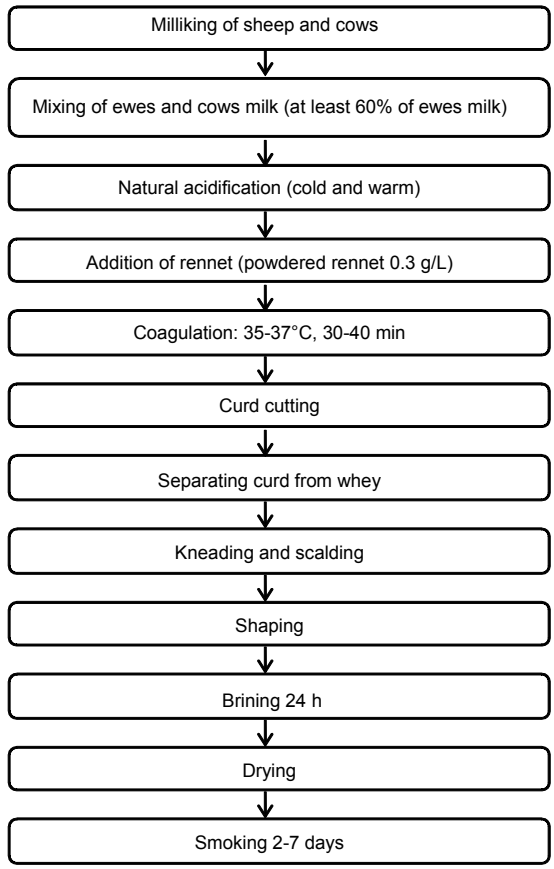

Fig. 1. Main steps of Oscypek preparation according to PDO regulations

Original Oscypek cheese can be produced only during sheep milking season that is from May till September, whereas the demand for Oscypek is all year long which leads to the production of its artifact – oscypek-like cheeses. The major course of Oscypek adulteration involve preparation of Oscypek by shepherds from only cow milk but still with maintained traditional manner or production of cheese by local dairy plants with a mechanised system and from pasteurised milk (either cows or ewes). Chemical, microbial, and sensory properties are very important for characterising and differentiating these labelled foods from similar foods without label. The instrumental characterization of the volatile fraction is known to be one of the factors differentiating traditionally made foods from its adulterated products [Setkowa et. al 2007, Mallia et. al 2005, Larráyoz et al. 2001]. As for Oscypek characterisation several data have been published concerning chemical composition such as: protein, water, fat, CLA and Vaccenic acid content, mineral components or microbial profile [Drożdż 1999, Borys et al. 2006, Reguła and Bonczar 2007] however, no volatile profile has been described.

The aim of this study was to compare volatile compound composition and sensory profiles of Oscypek, Polish traditional ewe cheese with oscypek-like cheeses.

EXPERIMENTAL

Material and methods. For sensory and volatiles analysis four different cheeses were compared: Oscypek cheese prepared according to PDO regulations and three oscypek-like cheeses: type "CM industry" – produced from pasteurised cow milk in dairy plant, type "EM-industry" – produced from pasteurised ewe milk in dairy plant and type "CM-shepherds" – produced from unpasteurised cow milk in shepherds huts. Oscypek cheese and CM-sheperds were collected from shepherds hut called "Bacówka u Kazka" located in the Leśnica village of the Podhale region which was the first shepherds hut in Poland to obtain PDO certificate. Type CM-industry were collected from the Mlekovita dairy plant placed in the Zakopane town and type EM-industry cheeses from the dairy plant located in Białka Tatrzańska.

Extraction method of volatiles. Isolation of volatiles was performed with PDMS/CAR/DVB fiber obtained from Supelco company and conditioned prior to analyses according to the manufacturer's recommendations. The extraction parameters were as follows: for each analysis 10 g of a sample was mechanically grated and placed in a 40 mL vials and spiked with 1 μg of internal standard: benzene d_6 and sealed with a crimp cap provided with a needle-pierceable PTFE/silicone septum. The sample vial was placed in a 50°C block heater for 10 min to equilibrate and then septum was pierced with SPME needle. The fiber was exposed to a headspace of the sample for 45 min and after extraction time fiber was retracted into a needle and transferred immediately to an injection port of a gas chromatograph and desorbed at 260°C for 5 min.

Analysis of volatiles. Gas Chromatography/Time of Flight Mass Spectrometry (GC/TOFMS). Compounds identification was performed using GCxGC-TOFMS system (Pegasus IV, Leco) equipped with: DB-5 MS (25 m × 0.2 mm × 0.33 μm) coupled to BPX-50 (1.3 m × 0.1 mm × 0.1 μm). The injector temperature was set to 280°C. During the injection the fiber was maintained for 5 min in the splitless mode for 1 min and than in the split mode (20:1). The operating conditions were the following: helium flow 0.8 mL/min, initial oven temperature 40°C (1 min), then 10°C/min to 250°C.

The GC/TOFMS transfer line was kept at 280°C. Mass spectra were collected at electron ionisation mode with ion source temperature at 220°C, mass range m/z 33-330, acquisition rate of 30 scans/s and detector voltage –1750 V. Identification of volatiles was performed by comparison of retention indices and mass spectra of eluting compounds to those of the NIST 05 library match. A mixture of n-alkanes (C_8-C_{20}) dissolved in hexane was supplied by Supelco (Bellefonte, PA, USA) for retention index determination. The calculation was done using Chroma TOF software (version 3.34). Semiquatification of compounds, presented as relative peak area (arbitrary units presented as the mean value of three determinations), was achieved using characteristic ions listed in Table 1.

Table 1. Volatile compounds identified in Oscypek and oscypek-like cheeses using SPME-GC/TOFMS method

Name	LRI	Quant ione	Oscypek	CM shepherds	CM industry	EM industry
1	2	3	4	5	6	7
Free fatty acids						
Acetic acid	623	60	0.7827 ±0.1005	1.0027 ±0.0667	1.0541 ±0.1060	0.1423 ±0.0144
Butanoic acid	793	60	0.2106 ±0.0212	1.7955 ±0.1699	0.7432 ±0.0810	0.0236 ±0.0068
Hexanoic acid	987	60	0.0816 ±0.0020	0.3657 ±0.0784	0.0471 ±0.0070	0.0109 ±0.0023
Octanoic acid	1 073	60	0.0033 ±0.0003	0.0193 ±0.0061	0.0042 ±0.0010	0.0015 ±0.0001
Esters						
Acetic acid methyl ester	577	74	0.0130 ±0.0008	0.0202 ±0.0026	0.0021 ±0.0001	0.0152 ±0.0026
Butanoic acid methyl ester	721	74	0.2776 ±0.0648	0.5433 ±0.2816	0.0062 ±0.0010	0.0583 ±0.0041
Hexanoic acid methyl ester	933	74	0.1761 ±0.0602	0.2153 ±0.0564	0.0050 ±0.0010	0.0170 ±0.0028
Octanoic acid methyl ester	1 136	74	0.0104 ±0.0030	0.0131 ±0.0043	0.0011 ±0.0001	0.0028 ±0.0004
Hexanoic acid ethyl ester	1 011	88	0.0075 ±0.0007	0.0071 ±0.0015	0.0015 ±0.0001	nd
Ketones						
2,3-butanedione	609	86	0.2481 ±0.0580	0.0102 ±0.0002	0.0480 ±0.0030	0.0840 ±0.0034
2,3-pentanedione	696	100	0.0203 ±0.0010	0.0042 ±0.0008	0.0020 ±0.0001	0.0022 ±0.0001
3-hydroxy-2-butanone	928	86	0.2077 ±0.0461	0.0734 ±0.1040	0.0331 ±0.0010	0.0130 ±0.0017
2-butanone	710	88	0.0256 ±0.0051	0.8352 ±0.0490	0.0351 ±0.0010	0.1030 ±0.0073

Table 1 – cont.

1	2	3	4	5	6	7
2-pentanone	613	72	0.0067 ±0.0006	0.0065 ±0.0004	0.0072 ±0.0180	0.0074 ±0.0006
2-heptanone	687	86	0.0250 ±0.0015	0.0243 ±0.0007	0.0110 ±0.0050	0.0075 ±0.0008
2-octanone	898	58	0.0162 ±0.0020	0.0026 ±0.0003	0.0031 ±0.0010	0.0009 ±0.0001
2-nonanone	1 007	58	0.0021 ±0.0002	nd	nd	nd
Aldehydes						
Acetaldehyd	540	44	0.1365 ±0.0162	nd	nd	nd
2-butenal	658	70	0.0078 ±0.0010	nd	0.0632 ±0.0010	0.0281 ±0.0007
3-methyl butanal	662	58	0.0068 ±0.0024	0.0235 ±0.0046	0.0150 ±0.0010	0.1618 ±0.0231
Hexanal	808	72	0.0169 ±0.0013	nd	nd	nd
2-hexenal	863	55	0.0021 ±0.0012	nd	nd	nd
Heptanal	912	70	0.0171 ±0.0066	nd	nd	nd
3-methyl 2-butenal	791	84	0.0013 ±0.0002	nd	0.0031 ±0.0001	nd
Furfural	842	96	0.1081 ±0.0080	nd	0.0351 ±0.0022	0.1058 ±0.0794
Nonanal	1 121	57	0.0031 ±0.0001	nd	nd	nd
Benzaldehyde	986	77	0.0113 ±0.0010	nd	0.0112 ±0.0013	0.0096 ±0.0014
Alcohols						
1-propanol	536	59	0.0019 ±0.0004	0.0245 ±0.0010	0.0061 ±0.0012	nd
Ethyl alcohol	551	45	1.4956 ±0.2383	0.4199 ±0.0313	1.2990 ±0.0890	0.0639 ±0.0013
2-butanol	736	55	0.0420 ±0.0034	0.6552 ±0.0154	0.0361 ±0.0051	0.0105 ±0.0009
3-methyl1-butanol	863	98	0.2282 ±0.0271	0.1494 ±0.0005	0.0161 ±0.0020	0.1442 ±0.0373
2-furanmethanol	798	47	0.0209 ±0.0007	0.1340 ±0.0079	0.1142 ±0.0180	nd
2,3-butanediol	770	55	0.0101 ±0.0027	nd	nd	0.0003 ±0.0001
1-pentanol	536	59	0.0514 ±0.0113	0.0351 ±0.0012	nd	0.0136 ±0.0001

Table 1 – cont.

1	2	3	4	5	6	7
Furans and furanones						
2(5H)furanone	1 017	69	0.0045 ±0.0008	0.0730 ±0.0078	0.0041 ±0.0014	0.0123 ±0.0013
2,5-dihydro-3,5-dimethyl 2-furanone	993	69	0.0036 ±0.0008	0.0524 ±0.0082	0.0230 ±0.0031	nd
3-methyl 2 (5H)-furanone	618	53	0.0162 ±0.0054	0.0361 ±0.0014	nd	0.0099 ±0.0012
5-methyl 2-furancarboxaldehyde	980	110	0.0135 ±0.0065	nd	0.1291 ±0.0058	nd
Phenols						
Phenol	994	94	0.2489 ±0.0554	0.6816 ±0.0621	0.1231 ±0.0214	0.2984 ±0.0251
2-methylphenol	1 072	108	0.0266 ±0.0053	0.0538 ±0.0079	0.0172 ±0.0022	0.0144 ±0.0016
3-methyl phenol	1 093	109	0.0252 ±0.0041	0.0866 ±0.0025	nd	0.0153 ±0.0022
2-methoxy phenol	1 110	109	0.0840 ±0.0194	0.1229 ±0.0176	0.1340 ±0.0180	0.0324 ±0.0052
2.3-dimethyl phenol	1 132	107	0.0024 ±0.0006	0.0031 ±0.0001	nd	0.0011 ±0.0001
4-ethyl-2-methoxy phenol	1 299	137	0.0056 ±0.0008	0.0028 ±0.0001	0.0054 ±0.0013	0.0030 ±0.0009
2-methoxy-4-methyl phenol	1 210	123	0.0249 ±0.0065	0.0134 ±0.0026	0.0291 ±0.0041	nd
Sulfur compounds						
Methanethiol	927	79	0.0006 ±0.0001	0.0007 ±0.0001	0.0021 ±0.0001	nd
Dimethyl disulfide	751	94	nd	0.0025 ±0.0001	nd	nd
Dimethyl sulfone	505	47	nd	0.0138 ±0.0004	nd	0.0038 ±0.0001
Dimethyl sulfide	586	76	0.0441 ±0.0033	0.0315 ±0.0051	0.0051 ±0.0001	nd
Terpenes						
α-pinene	956	93	0.0042 ±0.0008	0.0052 ±0.0015	0.0041 ±0.0001	nd
α-phellandrene	1 057	93	0.0048 ±0.0009	0.0062 ±0.0002	0.0020 ±0.0001	0.0004 ±0.0001

LRI – retention Indices on RTX-5 + BPX-50 columns.
Quant Ions – ions used for semi-quantification.
nd – not detected.
Results are presented as relative peak area (peak area of the compound divided by peak area of internal standard benzene d6) with standard deviation value. Samples are described in "material and methods" part.

Descriptive sensory analysis. Sensory analyses were performed by a 10-member panel experienced in descriptive analysis. The odour profiling analysis of all samples was run in triplicates (three sessions) proceeded by an introductory session. Eight flavour attributes were scaled on a 10 cm linear scale anchored on both sides for the intensity of attributes as "none" and "very strong". The odour descriptors were chosen according to the published paper Barcenas et al. [1999]. The 20 g cheese samples in a form of 1 cm^3 cubes were presented to the panelists in 50 mL glass containers. The results from linear scale were converted into numerical values for data analysis. Mean, variance and standard deviation were calculated for all attributes of each sample, for each session separately and across all three sessions. The obtained data were counted from 30 replicates and after statistical interpretation by multivariate procedure presented as graphic projection of Principal Component Analysis (PCA).

RESULTS AND DISCUSSION

Sensory profile analysis. Four cheeses: original Oscypek and three oscypek-like cheeses: CM-industry, EM-industry and CM-shepherds were presented for sensory descriptive analysis and evaluated by a 10 member sensory panel, who assessed odour and taste of the obtained cheese samples. Results are presented in Figure 2. All analysed cheese samples were compared with each other in relation to all used flavour attributes. In a first case, sensory analysis revealed significant differentiation of flavour profiles of analysed samples (Fig. 2). The first principal component (PC1) contained 58% of the variance in the data, and the second principal component (PC2) contained 34%. Most of flavour descriptors were strongly detected in the analysed samples and differentiated them into three groups. From presented data it could be concluded that original Oscypek had uppermost developed flavour of all cheese samples with highest scores obtained for five descriptors. It was separated from oscypek-like cheeses with strongly detected notes such as pungent, milky, brine, butyric and smoky. Last two descriptors were also strongly perceived in CM-shepherds cheese which is made from unpasteurised cow milk in shepherd huts. Last group consisted of CM-industry and EM-industry cheeses which were judged with the weakest notes for most descriptors except toasty flavour.

Volatile profile analysis. Table 1 reports volatile compounds identified in Oscypek and oscypek-like cheeses. All compounds were semi-quantified and the results were presented as relative peak area (peak area of the compound divided by peak area of internal standard benzene d_6). They do not represent absolute amount of a compound present in Oscypek cheese but they were calculated and used only for observation of differences between cheese samples. Each measurement was repeated three times. SPME-GC/TOFMS method revealed 51 volatile compounds representing nine chemical groups such as: free fatty acids, esters, ketones, alcohols, aldehydes, furans and furanones, phenols, sulfur compounds and terpenes.

Free fatty acids which represent major group of volatiles in cheese not only develop specific aroma but they also are precursors for other important volatiles such as methyl ketones, alcohols, lactones and esters. Due to their low aroma thresholds, short and medium-chain FFA are considered to be important contributors to the flavour of many cheese types. Their role is quite different depending on cheese variety, they give the main note to the flavour of aged Italian cheese but they are only background components

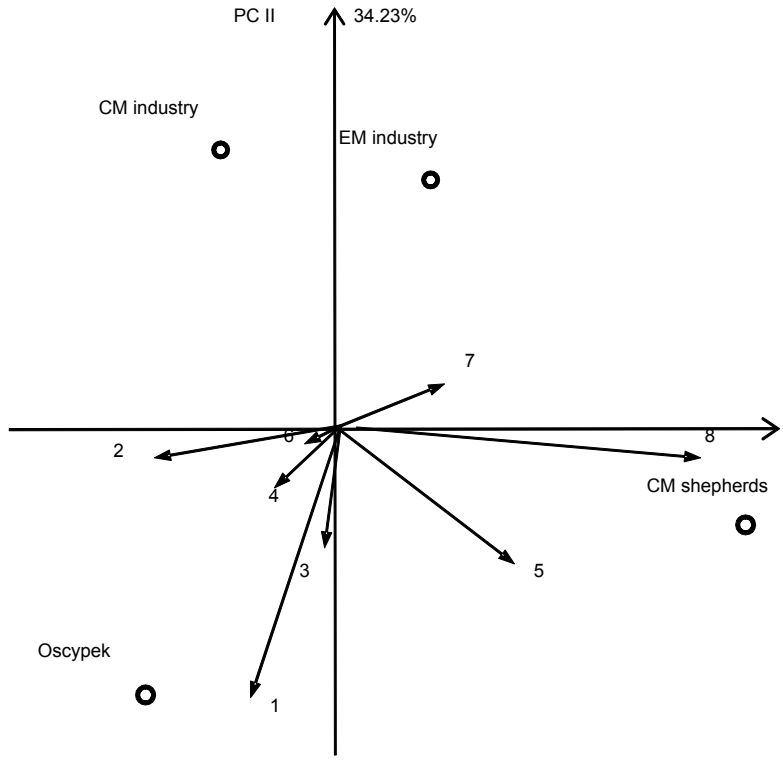

Fig. 2. PCA plot of flavour profile data of four cheese samples: original Oscypek and three oscypek-like cheeses: CM-industry, EM-industry and CM-shepherds (explanation in the text): 1 – pungent, 2 – milky, 3 – brine, 4 – buttery, 5 – butyric, 6 – rennet, 7 – toasty, 8 – smoky

in other type of cheeses such as Cheddar. Acetic acid contributes a mild to strong sharp-vinegar note, butanoic acid gives characteristic cheesy sharp aroma and hexanoic acid is usually perceived as a very mild to strong sharp, goat-like smell [Moio and Addeo 1998, Larráyoz et al. 2001]. Esters represent a minor group of volatiles identified in all cheeses. They usually give a pleasant flower and fruit notes to aroma of cheese and diminish sharpness of short chain free fatty acids and they are developed during esterification of fatty acids and alcohols [Bosset and Liardon 1984]. Alcohols usually bring sweet, flovery flavour to cheese aroma and they may derive from lactose degradation reduction of methyl ketones or amino acids metabolism [Molimard and Spinnler 1996]. It is hard to describe odour notes of aldehydes because it very much depends on their concentration in the product, i.e. 3-methyl butanal is characterised as spicy, cocoa, apple-like, cheese, green, malty or harsh [Corrëa Lelles Nogueira et al. 2005]. They are also known to be powerful odorants due to their low odour threshold values. The straight-chain aldehydes are formed during β-oxidation of unsaturated fatty acids. The branched chain aldehydes can be produced from amino acid degradation [Belitz et al. 2004]. Methyl ketones are recognised as key odorants of mould ripened cheese. Their flavour notes are described as fruity floral for 2-pentanone, 2-nonanone and

2-undecanone and spicy, musty for 2-heptanone or resin for 2-octanone. On the other hand di-ketones such as diacetyl bring nice buttery flavour. Furans and furanones are usually formed by thermal degradation of fructose in presence of amines and amino acids via Maillard reaction and are associated with roasted and caramel notes [Belitz et al. 2004]. Phenols are known to play important role in the flavour of cheeses due to their very low perception thresholds, 1 ppb or less. Some authors describe them as characteristic compounds of ewes' cheeses [Moio et al. 1993]. Their sensory quality ranges from sharp, medicinal, sweet, aromatic to smoky, plastic and unpleasant sheep-yard note. Phenols are constituents of a smoke and they deposit on the surface of cheese during smoking and then penetrate into the food. Terpenes belong to potentially feed derived compounds in cheese as they usually are brought to cheese flavour as milk constituents [Corrëa Lelles Nogueira et al. 2005].

In the presented study Oscypek cheese was represented by the largest number of volatiles identified – 51, which shows a relationship with sensory analysis where Oscypek has been described as a cheese with mostly developed flavour bouquet. In oscypek--like cheeses there were big differences in volatile and sensory profiles. For CM-shepherds cheese, which is made by shepherds from only cow milk but using the same way of preparation as is used for traditional Oscypek sensory analysis showed strongly detected butyric, toasted and smoked notes which could be associated with high amount of butanoic acid, 3-methyl butanal and phenolic compounds respectively. It could be noticed that both cheeses: Oscypek and CM-sheperds, which are made from unpasteurised milk using natural microflora and traditional technology of production which includes hand processing, application of wooden tools and natural preservation methods such as brining and smoking in shepherds huts had larger amount of volatiles identified and also had more developed aroma. This also agrees with published data, which stated that industrially made cheeses are known to be less developed in flavour than artisan foods [Barron et al. 2005, Centeno et al. 1999].

CONCLUSIONS

The rapid headspace SPME-GC/TOFMS analytical method was used for the determination of volatile profile of Oscypek and oscypek-like cheese. The differences showed in volatile fraction of original Oscypek cheese and adulterated ones provide possibility of employing SPME-GC/TOFMS technique to find adulteration in PDO labelled Oscypek. In further studies authors are planning to employ statistical evaluation based on PCA (principal component analysis), linear discriminant analysis (LDA) and SON (self organising maps) with combination of volatile analysis to confirm authenticity of PDO labelled Oscypek within its adulterated kind available on the Polish market.

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PORÓWNANIE ORYGINALNEGO OSCYPKA Z JEGO PODRÓBKAMI NA PODSTAWIE PROFILU ZWIĄZKÓW LOTNYCH ORAZ OCENY SENSORYCZNEJ

Wstęp. Celem pracy było porównanie profilu związków lotnych oryginalnego oscypka z serami typu oscypek z wykorzystaniem metody SPME-GC/TOFMS jako możliwość do zastosowania w rutynowych badaniach autentyczności polskich serów o Chronionej Nazwie Pochodzenia (ChNP).

Materiał i metody. W analizie związków lotnych i ocenie sensorycznej wykorzystano oryginalne oscypki wytwarzane z mleka owczego według rozporządzenia PDO oraz trzy sery typu oscypek: „CM industry" otrzymywane z pasteryzowanego mleka krowiego metodą przemysłową, „EM-industry" otrzymywane z pasteryzowanego mleka owczego me-

todą przemysłową oraz „CM-shepherds" wytwarzane z niepasteryzowanego mleka krowiego metodą tradycyjną. Izolacja związków lotnych została przeprowadzona z zastosowaniem włókna PDMS/CAR/DVB. Identyfikację związków lotnych przeprowadzono na chromatografie gazowym sprzężonym ze spektrometrem czasu przelotu.
Wyniki. Analiza fazy nadpowierzchniowej metodą SPME-GC/TOFMS pozwoliła na identyfikację 51 związków w oryginalnym oscypku i serach typu oscypek, które należały do dziewięciu grup chemicznych: wolnych kwasów tłuszczowych, estrów, ketonów, alkoholi, aldehydów, furanów i furanonów, związków fenolowych i siarkowych oraz terpenów. Stwierdzono, że oryginalny oscypek cechował się największą liczbą zidentyfikowanych związków lotnych w porównaniu z serami typu oscypek, co również znalazło odzwierciedlenie w wynikach analizy sensorycznej. Oryginalny oscypek został oceniony jako ser o bogatszym bukiecie aromatycznym. Dodatkowo zauważono, że sery wytwarzane z mleka niepasteryzowanego metodą tradycyjną, zarówno owczego, jak i krowiego, cechują się wyraźniejszym i bogatszym profilem związków lotnych.
Podsumowanie. Wykazane różnice w profilach związków lotnych pomiędzy oryginalnym oscypkiem a serami typu oscypek mogą być wykorzystane jako wskaźniki zafałszowań serów należących do grupy produktów o ChNP.

Słowa kluczowe: Oscypek, związki lotne, zafałszowania, produkty o ChNP, chromatografia gazowa, spektrometria mas, jakość

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