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INVESTIGATION OF THE APPLICABILITY OF SPME-GC/MS TECHNIQUE AND PRINCIPAL COMPONENT ANALYSIS IN THE EVALUATION OF A VOLATILE FRACTION OF BLUE-VEINED CHEESES

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Key words: blue-type cheeses, volatile fraction, HS-SPME, PCA

An object of the research were five blue-veined cheeses purchased at local supermarkets. Additionally, a cheese being a combination of a blue-type and another rennet coagulated cheese was evaluated. Extraction of the volatile components was performed by means of SPME in the headspace mode (HS-SPME) and followed by GC/MS separation and identification of the compounds isolated. Then, PCA was applied to the data obtained. The dimensionality of the data set was reduced from 27 volatiles to only four principal components (PCs), which accounted for about 93% of the total variance and allowed the classification of the samples studied.

INTRODUCTION

Changes occurring during the ripening of blue-veined cheeses are dominated by enzymatic breakdown of basic milk constituents such as proteins, fats, lactate and citrate. Proteolytic and lipolytic enzymes either produced by *Penicillium roquefoerti* and lactic acid bacteria or introduced with the milk and rennet paste are responsible for the release of compounds such as free fatty acids or amino acids and their transformation into the compounds affecting various, *e.g.* sensory, characteristics of the final product obtained. Volatiles formed are proved to have a strong influence on aroma and flavour development of the cheese. Mechanisms of changes undergoing during blue-type cheeses maturation process are described in detail at Gripon [1987] and Fox *et al*. [2003]. There are also several reviews available dealing with the subject [Yvon & Rijnen, 2001; Marilley & Casey, 2004].

Recently, several articles have been published treating of the blue-veined cheeses volatile and/or aroma fraction analysis and a wide range of instrumental methods have been applied for this purpose [Dartey & Kinsella, 1971; Gonzalez de Llano *et al*., 1990; de Frutos *et al*., 1991; Moio *et al*., 2000; Lawlor *et al*., 2003; Frank *et al*., 2004; Trihaas *et al.*, 2005]. Of sample preparation techniques used for food volatiles isolation solid phase microextraction (SPME) is becoming increasingly popular [Arthur & Pawliszyn, 1990; Zhang *et al*., 1994; Kataoka *et al*., 2000]. It has been successfully applied to the blue-type cheeses volatile fraction evaluation [Frank *et al*., 2004].

In the case of food products, volatiles analysis usually results with complex data matrix. The level of complexity of data set obtained makes it necessary for researchers to use multivariate statistical methods for data interpretation. Among various chemometrical approaches proposed principal component analysis (PCA) deserves special attention. It was proved to be a useful tool for blue-veined cheeses volatiles analysis [Lawlor *et al*., 2003; Trihaas *et al*., 2005].

Although several papers have been published concerning blue-type cheeses volatiles, majority of them treats of the most popular varieties such as Gorgonzola or Danish Blue, and there is none dealing with Polish products of this type. The present study was aimed to fill this gap. The goal of the research was to investigate whether SPME-GC/MS technique "coupled" with PCA may be successfully used to evaluate qualitative and quantitative differences in volatiles composition of blue-veined cheese varieties available in Poland, including the two Polish products.

MATERIALS AND METHODS

An object of the study were five blue-type cheese varieties and the one being a sort of combination of the blue-veined and another rennet coagulated cheese. The cheeses were purchased at Warsaw supermarkets and kept refrigerated prior to analysis.

Samples of 5 g of cheese were weighed into a pestle and pounded with 10 g of dearomatised dry NaCl with 0.3 μL of internal standard (the internal standard was valeric acid methyl ester added manually with a Hamilton syringe). A 5 g portion of the mixture obtained was transferred into a glass jar and shut with a lid. Then, equilibration (5 min, 20°C) and extraction (10 min, 20°C) steps followed. Isolation of the

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volatiles compounds was performed by introducing CAR/PDMS/DVB fiber into the headspace of the sample. The fiber was subsequently transferred into the injection port of gas chromatograph coupled with mass spectrometer (QP2010, Shimadzu). GC oven temperature was programmed as follows: 2 min at 40°C, increased at 4°C/min to 180°C and kept at 180°C for 6 min. Temperatures of the injection port, the interface and the ion source were 220°C, 200°C and 190°C, respectively. The split ratio was 1:3.9. A capillary column ZB-WAX (30.0 m length, 0.25 mm internal diameter and 0.25 μm film thickness, Phenomenax) was used for the separation. The carrier gas was helium applied at a flow rate of 0.75 mL/min. A mass spectrometer worked in a total ion current mode and the identification was by comparison of the spectra obtained with those from libraries (Wiley7N2, NIST147, FFNSC Flavor & Fragrance Library with Linear Retention Index). Each cheese was analysed in triplicate and the peak area of every compound was divided by the internal standard's peak area – average of the three replicates gave the so-called mean relative peak areas.

PCA was applied to the compounds present in at least three out of six cheeses analysed in order to facilitate data interpretation process and visualize relationships between compounds detected. The data were standardized to 0 mean and 1 standard deviation and the correlation matrix was obtained. Then, eigenvalues and eigenvectors of the matrix were calculated following the instructions stated in Dubrow [1978], Morrison [1990] and Jajuga [1993].

RESULTS AND DISCUSSION

A total of 49 headspace constituents were identified in the cheeses evaluated. The number of components detected varied between individual cheeses from 22 (Bleu d'Auvergne) to 33 (Dorblu). In Rokpol, Fourme d'Ambert, Duo Blue and Lazur 26, 27, 28 and 31 volatiles were identified, respectively. All the compounds identified in the samples studied are listed in Tables 1 and 2. Assignment into chemical classes is performed and the presence in individual varieties is marked.

Alcohols formed the largest group of volatiles detected. Of them, only 2-butanol, 4-methyl-2-pentanol, 1-heptanol, 6-methyl-5-hepten-2-ol, 2-furanmethanol and 2-(2-butoxyethoxy)-ethanol were present in the samples of just one or two of the cheeses investigated (Table 1). Methyl ketones comprised the second most abundant chemical group of headspace constituents identified. Most of them, *e.g.* 2-pentanone, 2-hexanone, 2-heptanone and 2-nonanone, were detected in every cheese studied (Table 2). The headspace of all the cheeses investigated contained free

TABLE 1. Volatile alcohols and fatty acids of the cheeses studied.

Components	Fourme d'Ambert	Duo Blue	Dorblu	Bleu d'Auvergne	Lazur	Rokpol
Alcohols						
2-Butanol						⩒
2-Methylpropanol	∨	∨	∨	∨	∨	∨
2-Pentanol	∨	∨	∨	∨	∨	⩒
1-Ethoxy-2-propanol	∨	∨	∨	∨	∨	∨
3-Methyl-1-butanol	⩒	∨	⩒	∨	∨	∨
4-Methyl-2-pentanol						∨
1-Pentanol	∨	∨	∨		∨	
2-Heptanol	∨	∨	∨	∨	∨	∨
1-Octen-3-ol			∨	∨	∨	
1-Heptanol			∨			
6-Methyl-5-hepten-2-ol			∨			
2-Ethyl-1-hexanol	∨	∨	∨			
2-Nonanol	∨	∨	∨	∨	∨	∨
1,3- or 2,3-Butanediol*	∨	∨			∨	∨
1,3- or 2,3-Butanediol**		∨	∨		∨	∨
2-Furanmethanol		∨				
2-(2-Butoxyethoxy)-ethanol					∨	
Fatty acids						
Ethanoic acid		∨				∨
2-Methylpropanoic acid	∨	∨	∨		∨	∨
Butanoic acid	∨	⩒	∨	∨	∨	∨
3-Methylbutanoic acid	∨	∨	∨		∨	∨
Hexanoic acid	∨	∨	∨	∨	∨	∨

∨ – compound identified and quantified; ⩒ – mean relative peaks area greater than 1; * – identification equivocal, retention time: 20.32; ** – identification equivocal, retention time: 21.46

fatty acids and esters (Tables 1 and 2). Butanoic, hexanoic and 3-methylbutanoic acids as well as isopentyl butanoate were found in each of the cheeses analysed. Of other compounds detected only *p*-methylanisole, methoxy-phenyloxime and butyrolactone seemed to be more abundant (Table 2).

The mean relative peak areas of 2-heptanone and 3-methyl-1-butanol exceeded 1 in Fourme d'Ambert cheese, but the presence of 2-pentanone, 2-heptanol, 2-pentanol and butanoic acid was also fairly noticeable. Fourme d'Ambert was the one of the cheeses investigated in which 4-methyl-2 .6-ditertbutylphenol was observed. The greatest mean relative peak areas of straight chain fatty acids (ethanoic, butanoic and hexanoic acids) detected compared to the other cheeses investigated were distinctive for Duo Blue cheese. In the volatile fraction of this variety 2-pentanone, 3-methyl-1-butanol, 2-heptanone and acetoin also seemed to play an important role. 2-Heptanone, 2-nonanone and 3-methyl-1-butanol, all having the mean relative peak areas greater than 1, were dominant volatiles of Dorblu samples. Headspace composition of this cheese was clearly distinguishable by the presence of 1-heptanol, 6-methyl-5-hepten-2-one and phenylethanol. Although neither 2-heptanone, 2-nonanone, 2-pentanone nor 3-methyl-1-butanol had mean relative peak areas greater than 1 they seemed to play a dominant role in the volatiles profile of Lazur cheese. 3-Methyl-1-butanol, 2-heptanone and 2-methylpropanol had the greatest mean relative peak areas of the volatiles of Bleu d'Auvergne cheese. The presence of dimethyl disulphide was a distinctive feature of these two varieties compared to the other cheeses studied. Volatiles profile of Rokpol cheese distinguished itself by the presence of 2-butanol and the lack of 2-methylpropanol and was dominated by 2-heptanone, 2-pentanone and 2-pentanol.

The results presented above confirm a dominant role of alcohols and methyl ketones in the volatile fraction of blue-veined cheeses and they stay in agreement with those previously published. Other authors reported that methyl ketones

TABLE 2. Volatile ketones, aldehydes, esters and other compounds of the cheeses studied.

Components	Fourme d'Ambert	Duo Blue	Dorblu	Bleu d'Auvergne	Lazur	Rokpol
Ketones and aldehydes						
2-Pentanone	∨	∨	∨	∨	∨	**∨**
2-Hexanone	∨	∨	∨	∨	∨	∨
2-Hydroxy-3-hexanone						∨
2-Heptanone	**∨**	∨	**∨**	∨	∨	**∨**
3-Hydroxy-2-butanone (acetoin)	∨	∨		∨	∨	∨
2-Octanone	∨		∨			
6-Methyl-5-hepten-2-one	∨	∨	∨		∨	
3-Hydroxy-2-pentanone		∨				
2-Nonanone	∨	∨	**∨**	∨	∨	∨
8-Nonen-2-one	∨	∨	∨	∨	∨	∨
2-Undecanone	∨	∨	∨	∨	∨	∨
Nonanal		∨				
Decanal					∨	
Esters						
Isobutyl butanoate	∨		∨	∨		
Ethyl hexanoate			∨			∨
Isopentyl butanoate	∨	∨	∨	∨	∨	∨
Isobutyl hexanoate			∨			
Isopentyl hexanoate			∨			
Other compounds						
o-Xylene					∨	
p-Methylanisole	∨		∨	∨	∨	∨
Methoxy-phenyloxime	∨			∨	∨	
Phenylethanol			∨			
4-Methyl-2,6-ditertbutylphenol	∨					
Dimethyl disulphide				∨	∨	
2-Ethoxypropane			∨		∨	
1-(2-Propenyloxy)-heptane					∨	
Butyrolactone		∨	∨	∨		∨

∨ – compound identified and quantified; **∨** – mean relative peaks area greater than 1

and alcohols form the largest chemical groups of volatiles of blue-type cheeses, with 2-heptanone, 2-nonanone as well as 2-heptanol and 2-nonanol being the most abundant [Gripon, 1987; Dartey & Kinsella, 1971; Gonzalez de Llano *et al*., 1990; de Frutos *et al*., 1991; Moio *et al*., 2000; Lawlor *et al*., 2003; Frank *et al*., 2004; Trihaas *et al*., 2005]. Also volatile fatty acids and esters were pointed to be important volatile components of many European blue-veined varieties. The fact that the number of aromatic and sulphur compounds, aldehydes and lactones detected was smaller compared to previous studies mentioned may be because different method of volatiles isolation was applied – of the authors mentioned only Frank *et al.* [2004] extracted volatiles by means of SPME (and extraction phase lasted for 16 hours).

PCA performed on the 27 of the volatiles present in the headspace of the cheeses studied allowed to reduce the dimensionality of the data set from 27 volatile compounds to four principal components (PCs) which accounted for 93.04% of the total variance. PCs loadings for the samples analysed are presented in Table 3.

PC1 accounted for 38.18% of the total variance and it differentiated Dorblu and Fourme d'Ambert cheeses from the other varieties studied (Table 3). PC2 accounted for 23.62% of the total variance. It clearly separated Lazur, Bleu d'Auvergne and Dorblu samples from the other three cheeses. Analysis of the correlation coefficients values of the first two PCs with the volatiles subjected to PCA showed that the differentiation according to PC1 and PC2 loadings was related to the abundance of compounds such as 1-ethoxy-2-propanol, 3-methyl-1-butanol, 2-nonanol, 2-undecanone, 8-nonen-2-one (PC1) as well as 2-pentanone, 2-hexanone, 2-heptanone, 2-pentanol, 2-heptanol, butyrolactone and 1-octen-3-ol (PC2) (data not shown). Hence, it was suggested that the two first PCs as "the components of methyl ketones, alcohols and esters" characterised the overall volatile profile of the cheeses studied.

PC3 accounted for 16.19% of the total variance. As "the component of straight chain fatty acids, methyl ketones and 2-alkonols" it separated Duo Blue and Fourme d'Ambert from the remaining varieties analysed. In Duo Blue as well as in Fourme d'Ambert samples straight chain fatty acids were fairly abundant.

PC4 accounted for 15.05% of the total variance. Separation according to PC4 was in relation with the abundance of *p*-methylanisole, methoxy-phenyloxime as well as 2-methyl-propanol, 1-ethoxy-2-propanol, 1-pentanol and 1-octen-3-ol. PC4 clearly segregated Fourme d'Ambert (noticeably positive PC4 loadings) and Dorblu (negative PC4 loadings) from the samples of the other cheeses investigated. It was described as "the component of aromatic compounds and alcohols other than 2-alkanols".

Analysis of the first four PCs values for Lazur and Bleu d'Auvergne showed that they were grouped into one cluster. This suggested some kind of similarity between these two varieties of volatiles profiles.

TABLE 3. The first four PCs loadings for the samples analysed.

PCs	Cheese samples		
	Dorblu		
PC1	3.03259	1.66637	4.04218
PC2	-2.01384	-2.33211	-2.41043
PC3	-1.36660	-0.79129	-1.48794
PC4	-3.13022	-2.26691	-3.87248
	Lazur		
PC1	-2.95633	-2.26974	-0.84194
PC2	-1.51849	-1.72992	-1.00259
PC3	-0.48531	-0.60038	-0.94741
PC4	1.53488	1.10406	0.26030
	Fourme d'Ambert		
PC1	2.23744	6.17345	6.44159
PC2	-0.47964	0.80136	0.03646
PC3	0.42787	1.45276	2.19922
PC4	2.94729	1.83998	3.03476
	Rokpol		
PC1	-1.40668	1.14703	-1.52964
PC2	3.40377	6.00400	4.42658
PC3	-2.03201	-3.26381	-1.16018
PC4	0.35981	-0.77058	0.15382
	Bleu d'Auvergne		
PC1	-2.69119	-2.76814	-2.69231
PC2	-1.90523	-2.12711	-1.73411
PC3	-0.52628	-0.65481	-0.51163
PC4	0.82834	0.80583	0.73028
	Duo Blue		
PC1	-3.10992	-2.58060	-1.89418
PC2	-0.26784	0.11663	2.73248
PC3	1.30733	2.40709	6.03339
PC4	0.26596	-0.46191	-3.36320

CONCLUSIONS

The results obtained confirm that methyl ketones, alcohols as well as fatty acids and esters play a leading role in the volatile fraction of blue-type cheeses.

PCA applied to the 27 of the volatiles identified allowed to perform a clear grouping of the samples analysed with only 7% loss of information. It was possible to reduce the dimensionality of the data set and obtain satisfactory classification of the cheeses studied.

Analysis of the qualitative composition of the volatile fraction clearly showed that Duo Blue samples distinguished themselves by greater amounts of straight chain fatty acids compared to the other cheeses investigated. This could be due to the specific character of this variety as it was a sort of a combination of a "traditional" blue-veined cheese and another rennet coagulated product. The separation of Duo Blue samples was confirmed by PCA results. PCA suggested also some kind of similarity between Lazur and Bleu d'Auvergne volatile profiles.

SPME-GC/MS technique "coupled" with PCA was proved to be a useful tool for analysis of the qualitative and quantitative differences between the volatile profiles of blue-veined cheeses available on the Polish market.

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OCENA MOŻLIWOŚCI ZASTOSOWANIA TECHNIKI SPME-GC/MS ORAZ ANALIZY METODĄ GRUP I CECH DO BADAŃ ZWIĄZKÓW LOTNYCH SERÓW Z PRZEROSTEM PLEŚNI

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Przedmiotem badania były próbki pięciu serów z przerostem pleśni zakupione w lokalnych supermarketach. Dodatkowo, analizie poddano ser będący swego rodzaju kombinacją sera z przerostem pleśni i innego sera podpuszczkowego. Ekstrakcję związków lotnych przeprowadzono techniką mikroekstrakcji z fazy nadpowierzchniowej (HS-SPME). Rozdziału i identyfikacji wyizolowanych związków lotnych dokonano techniką GC/MS. Uzyskane dane poddano obróbce metodą analizy grup i cech. Udało się zredukować wymiarowość przestrzeni danych z 27 związków lotnych do czterech składowych głównych, które wyjaśniały ogółem ok. 93% zmienności całkowitej i umożliwiły klasyfikację badanych próbek.