POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 31(1): 75–87, Y. 2016

EFFECT OF STORAGE OF ROKPOL CHESSE ON VOLATILE COMPOUNDS PROFILES

***Piotr Karpiński*[1], *Bartosz Kruszewski*[2], *Magdalena Sobocińska*[1], *Marta Łątkowska*[1], *Małgorzata Gomółka-Pawlicka*[3], *Mieczysław W. Obiedziński*[1]**

[1] Department of Biotechnology, Microbiology and Food Evaluation, Warsaw University of Life Sciences
[2] Department of Fruit and Vegetable Product Technology, Institute of Agricultural and Food Biotechnology in Warsaw
[3] Department of Veterinary Public Health University of Warmia and Mazury in Olsztyn

K e y w o r d s: mold cheese, HS-SPME, volatile compounds, principal component analysis, cluster analysis.

A b s t r a c t

The aim of this study was to investigate changes of volatile compounds in Rokpol blue cheese during storage under different temperature conditions (4°C, 25°C, 35°C). Headspace solid-phase microextraction (HS-SPME) was used to isolate volatile compounds from the matrix and GC/MS was used for compounds separation and identification. Received aroma profiles were showed in the analyzed cheese and statistical analysis were done based on the identified groups of compounds. Results were interpreted on the basis of principal component analysis and cluster analysis. The dominant group of compounds represented ketones. The largest decrease in quality in the profile of volatile compounds was observed during storage at 25°C. Profile of volatile compounds remains similar during the week in 4°C and two days at 25°C.

WPŁYW WARUNKÓW PRZECHOWYWANIA NA ZMIANY W PROFILU ZWIĄZKÓW LOTNYCH SERA TYPU ROKPOL

***Piotr Karpiński*[1], *Bartosz Kruszewski*[2], *Magdalena Sobocińska*[1], *Marta Łątkowska*[1], *Małgorzata Gomółka-Pawlicka*[3], *Mieczysław W. Obiedziński*[1]**

[1] Katedra Biotechnologii, Mikrobiologii i Oceny Żywności, Szkoła Główna Gospodarstwa Wiejskiego w Warszawie
[2] Zakład Technologii Przetworów Owocowych i Warzywnych, Instytut Biotechnologii Przemysłu Rolno-Spożywczego w Warszawie
[3] Katedra Weterynaryjnej Ochrony Zdrowia Publicznego, Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: ser pleśniowy, HS-SPME, związki lotne, analiza składowych głównych, analiza skupień.

Address: Piotr Karpiński, Department of Biotechnology, Microbiology and Food Evaluation, Division of Food Quality Evaluation, University of Life Sciences, ul. Nowoursynowska 159, 02-787 Warsaw, phone: +48 (86) 215 54 94, e-mail: pkarpinski@wsip.edu.pl

Abstrakt

Celem pracy było zbadanie zmian profili związków lotnych w serze pleśniowym Rokpol w czasie przechowywania w różnej temperaturze (4°C, 25°C, 35°C). W celu izolacji związków lotnych z matrycy sera wykorzystano technikę mikroekstrakcji z fazy nadpowierzchniowej (HS-SPME), natomiast do rozdziału i identyfikacji zastosowano chromatografie gazową sprzężoną ze spektrometrią mas (GC/MS). Przedstawiono otrzymane profile związków lotnych w badanym serze oraz dokonano analizy w oparciu o grupy zidentyfikowanych związków, a wyniki zinterpretowano w oparciu o analizę składowych głównych oraz analizę skupień. Dominującą grupę związków stanowiły ketony. Największy spadek jakościowy w profilu związków lotnych odnotowano podczas przechowywania w temperaturze 25°C. Profil związków lotnych pozostaje podobny podczas tygodniowego przechowywania w lodówce i dwóch dni w temperaturze 25°C.

Introduction

The formation of unique characteristics of cheese is conditioned on the production process, including the type of milk used, humidity, NaCl content, pH of the product, a kind of used starter cultures and secondary microorganism as well as production stages. All these components have a significant impact on the biochemical changes occurring during the ripening process and future storage of cheese (FOX and MCSWEENEY 2004).

Milk fat is essential for proper formation of cheese flavor. Furthermore, the fat content affects microstructure, biochemical changes, production efficiency, rheological and textural properties of the cheese. The last properties influence how fast are the flavor compounds from the matrix of the cheese released (GUINEE and MCSWEENEY 2006).

Studying the formation of flavor compounds is extremely important from the point of view of cheese products production, ripening inspection and ways of accelerating the ripening time of cheese. Moreover it helps to avoid the appearance of any foreign smell in the cheese. The main biochemical changes occurring during maturation is glycolysis, lipolysis and proteolysis. Subsequent changes include the metabolism of compounds that arose as the result of major transformations (FOX et al. 1995).

Volatile compounds of food constitute a complicated system of analytes that often occur in trace amounts (ng/kg of product). However, from the point of view of the product flavor, not only the content of individual compounds is important, but also their sensory detection threshold. It is defined as the lowest concentration level, which allows the consumer to smell a compound (JELEŃ 2004, SURBURG and JOHANNES 2006).

The aroma of cheese is affected by the variety of groups of compounds including e.g. alcohols, aldehydes, ketones, esters and lactones. The alcohols may be generated during cheese maturation in the metabolism of lactose and amino acids, the reduction of ketones or acids degradation (eg. linoleic and

linolenic acids). In the case of mildew cheeses the presence of 1-octen-3-ol is related to the metabolism of the mold *Penicillium*. Alcohols that constitue from changes of branched and aromatic amino acids and that influence cheeses aroma are e.g.: 3-methyl-butanol, 2-methyl-butanol, 2-methylpropanol, phenylethanol and tryptofol (MOLIMARD and SPINNLER 1996, YVON and RIJNEN 2001, CURIONI and BOSSET 2002, MARILLEY and CASEY 2004, VITOVA et al. 2006).

The substrate for the formation of aldehydes are amino acids produced during proteolysis. They are considered as transitional compounds because they are rapidly reduced to primary alcohols or oxidized to the suitable acids. The compounds that are often found in cheese and affecting their odor are: 2-methylpropanal, 2-metylobutanal and 3-metylobutanal (YVON and RIJNEN 2001, CURIONI and BOSSET 2002, MARILLEY and CASEY 2004).

The aim of the study was to research qualitative and quantitative changes in the profile of volatile compounds in Rokpol cheese, caused by different storage temperature and time.

Material and Methods

The research material was Rokpol cheese – Polish cheese veined throughout with the blue mold. It is patterned on the French cheese Roquefort, in which the characteristic taste and appearance is achieved by fungi *Penicillium roqueforti*. The cheese was purchased in one of Warsaw's supermarkets immediately after delivery. Then it was cut into pieces of the same size and vacuum packed. The cheese samples that was the control samples were frozen at -18°C immediately after packaging (samples marked as „0"). Further samples were placed in a refrigerator at a temperature of 4°C ± 2°C (labeled as „L"). Some were incubated at 25°C ± 2°C and the last were incubated at 35°C ± 1°C (denoted by the letters „P" and „C"). Samples were taken from the refrigerator every 7 days, while samples stored in 25°C and 35°C were collected every 24 hours. The figure accompanying the letter of the associated conditions meant another day or a week of storage. Immediately after taking each sample, it was frozed and stored at -18°C until analysis.

The procedure of sample preparation for analysis involved cheese grating with a fine mesh. Than 3 g of cheese were weighted to 20 ml vial and 1fl of internal standard solution (trans-2-decanal 0.067 μl/ml) was added. After sealing the vial, the sample was incubated at 40°C for 20 minutes. The volatiles were then extracted by headspace microextraction using SPME fiber type CAR/PDMS/DVB at 40°C for 20 minutes. The conditions for conditioning and extraction were determined experimentally. Volatiles were desorbed from the fiber in the injector chamber for 3 minutes at 220°C.

Chromatographic analysis was performed by gas chromatography coupled with mass spectrometer GCMS-QP2010S (Schimadzu). The column used was non-polar ZB-5ms (phase 5%-phenyl-95%-dimetylopolisiloksan arylene) with dimensions of 30 m x 0.25 mm x 0.25 mm. The temperature of the chromatography oven was programmed as follows: isothermal 40°C for 10 minutes, then the ramp rate of 4°C/min to 220°C isothermal for 5 minutes. The carrier gas was helium with a flow rate of 1.1 cm^3/min and a constant linear velocity. Data collection was performed in sweep 40-300 m/z, using the ionization energy of 70eV.

In order to calculate the retention indexes of the volatile compounds the mixture of n-alkanes C7 ÷ C30 was used (Sigma-Aldrich). Identification of volatiles was carried out on the basis of the mass spectra library WILEY7N2, NIST147 and NIST2008 and Kovats retention indexes which are available online (The Pherobase).

Statistical analysis was performed using Statistica 10.0. One-way analysis of variance (ANOVA) at the significance level $p \leq 0.05$. To evaluate the differences between mean values Tukey HSD test was used. In order to illustrate the differences in the volatile profiles of tested samples and because of the amount of compounds identified, obtained data were statistically analyzed by PCA (Principal Components Analysis). The results have been supplemented by CA (Cluster Analysis).

Results and Discussion

In the tested Rokpol samples a total of 37 of volatile compounds belonging to six chemical groups were identified: aldehydes (1), alcohols (9), fatty acids (5), esters (13), ketones (7), hydrocarbons (2).

Ketones, esters, alcohols and acids had the largest share in the volatile fraction of the control sample. Hydrocarbons were present at a low level and aldehydes were not detected at all. Additionally there were identified compounds commonly present in the cheese mold, such as 2-pentanol, 2,3-butanediol, 2-heptanol, 1-octen-3-ol, 2-pentanone, 3-hydroxy-2-butanone, 2-heptanone, 8-nonene-2-one and 2-nonanone. The volatile compounds typical for mould cheeses are fatty acids containing up to 10 carbon atoms and their methyl and ethyl esters. Those were also identified in the tested samples.

The largest share in the headspace phase constituted ketones such as 2-nonanone (with floral, fruity scents), and 2-heptanone (with mold, sweet and musty scents). Moreover, a large share in volatile fraction had also other compounds such as 2-heptanol, butanoic acid and hexanoic acid, as well as the ethyl esters of these acids. The group of compounds present in all samples at

a constant level irrespective of the conditions and storage time were: 2-pentanol, 2-nonanol, ethyl esters of butanoic acid and hexanoic acid and 2-undecan. The obtained volatile compounds profile of Rokpol cheese was consistent with the results obtained by other authors (MOLIMARD and SPINNLER 1996, CURIONI and BOSSET 2002, FRANK et al. 2004, TRIHAAS et al. 2005, BZDUCHA and OBIEDZIŃSKI 2006).

In the samples stored for one week under refrigerated conditions there was noticeable significant decrease in the intensity of all detected compounds as compared to the control sample. During following weeks of storage there was observed an increase in the intensity of synthesis of esters and acids, which finally after 4 weeks of storage had the largest share in the volatile fraction (Figure 1).

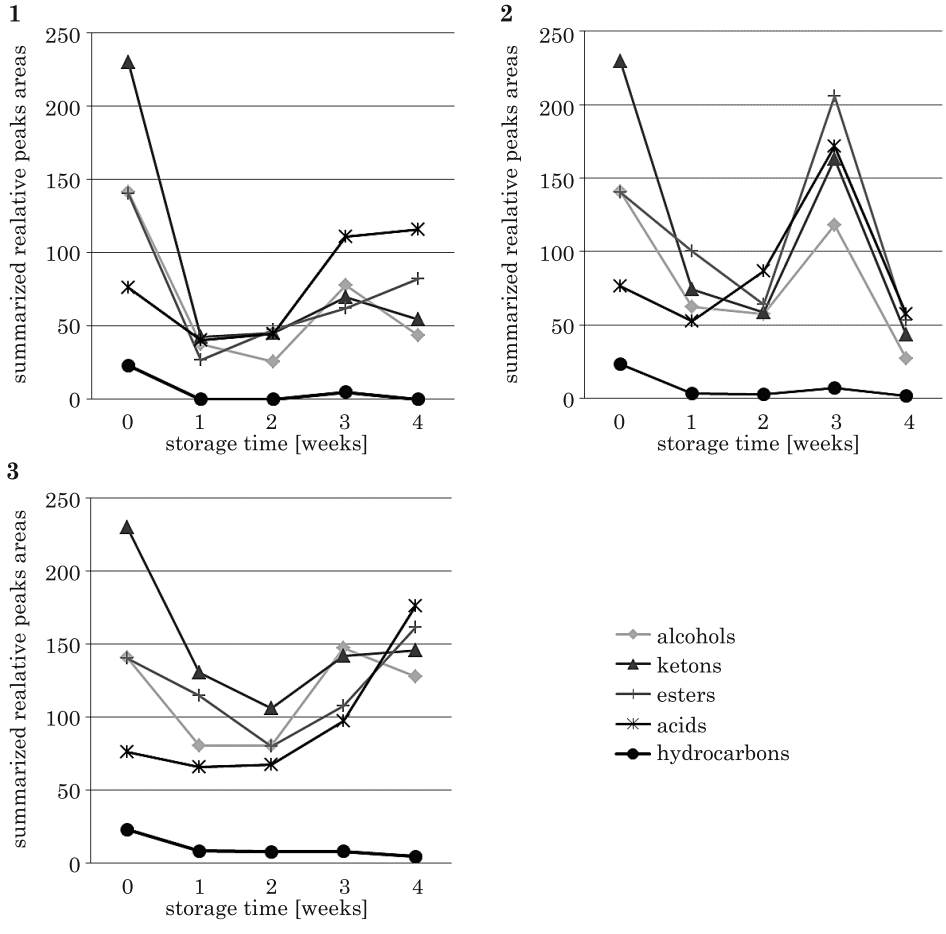

Fig. 1., 2. and 3. Relative peak areas changes of particular compound groups during different storage conditions: refrigerated (1), 25°C (2), 35°C (3)

Table 1

Content of volatile compounds in investigated cheese samples [average relative peaks areas in relation to the standard relative peak area (trans-2-decenal)]

Compound name		RI	Control	Refrigerated (4°C)				Temperature 25°C				Temperature 35°C			
			after purchase	1 week	2 week	3 week	4 week	1 day	2 day	3 day	4 day	1 day	2 day	3 day	4 day
1		2	3	4	5	6	7	8	9	10	11	12	13	14	15
Alcohols	2-pentanol	734	$10{,}1^{a}$ +4,9	$2{,}1^{a}$ +0,1	$1{,}3^{a}$ +0,2	$5{,}0^{a}$ +0,6	$3{,}0^{a}$ +0,2	$5{,}2^{a}$ +1,2	$4{,}3^{a}$ +1,4	$10{,}6^{a}$ +8,7	$2{,}3^{a}$ +0,3	$6{,}5^{a}$ +0,6	$5{,}0^{a}$ +0,6	$11{,}3^{a}$ +4,5	$9{,}0^{a}$ +0,4
	3-methyl-1-butanol	755	$11{,}6^{a}$ +4,8	$1{,}7^{b}$ +0,1	$0{,}8^{b}$ +0,1	$3{,}1^{b}$ +0,5	nd	$3{,}8^{b}$ +0,9	$1{,}9^{b}$ +0,5	$4{,}6^{a,b}$ +3,6	$1{,}0^{b}$ +0,1	$4{,}1^{b}$ +0,7	$3{,}8^{b}$ +0,2	$5{,}9^{a,b}$ +2,0	$5{,}1^{a,b}$ +0,1
	2,3-butandiol	788	$2{,}4^{a,b}$ +0,8	$0{,}8^{b,c}$ +0,1	nd	$1{,}7^{a,b,c}$ +0,5	$2{,}0^{a,b,c}$ +0,1	nd	nd	nd	nd	nd	nd	$3{,}6^{a}$ +1,7	$3{,}3^{a}$ +0,1
	1,3-butandiol	793	$5{,}8^{a,b}$ +1,0	$2{,}5^{b}$ +0,4	$4{,}5^{a,b}$ +1,1	$4{,}8^{a,b}$ +2,1	$9{,}9^{a}$ +0,4	$4{,}0^{a,b}$ +2,0	$2{,}1^{b}$ +0,7	$5{,}3^{a,b}$ +4,6	$1{,}5^{b}$ +0,7	$4{,}4^{a,b}$ +0,9	$4{,}2^{a,b}$ +0,5	$7{,}5^{a,b}$ +2,9	$7{,}1^{a,b}$ +0,9
	1,3-propandiol	831	nd	$0{,}8^{a,b}$ +0,1	nd	$2{,}0^{a,b}$ +0,6	nd	$2{,}2^{a,b}$ +1,4	$2{,}2^{a,b}$ +0,5	$3{,}2^{a}$ +1,2	$1{,}0^{b}$ +0,5^{a}	$2{,}2^{a,b}$ +0,6	$1{,}8^{a,b}$ +0,3	$2{,}2^{a,b}$ +1,5	nd
	2-heptanol	902	$73{,}2^{a}$ +17,2	$13{,}5^{c,d}$ +0,1	$7{,}5^{d}$ +0,5	$25{,}6^{b,c,d}$ +7,7	$10{,}8^{c,d}$ +0,3	$26{,}1^{b,c,d}$ +7,3	$20{,}2^{c,d}$ +4,0	$39{,}1^{a,b,c,d}$ +26,1	$12{,}0^{c,d}$ +3,0	$37{,}8^{a,b,c,d}$ +0,7	$32{,}0^{a,b,c,d}$ +1,6	$64{,}1^{a,b}$ +15,3	$50{,}7^{a,b,c}$ +11,5
	1-octen-3-ol	980	nd	nd	nd	nd	nd	0,8 +0,3^{a}	nd	nd	nd	nd	nd	nd	nd
	2-octanol	1002	nd	$6{,}8^{b}$ +0,1	nd	$12{,}2^{a,b}$ +1,5	nd	nd	$8{,}4^{a,}$ +0,3	$26{,}3^{a}$ +16,2	nd	nd	$11{,}3^{a,b}$ +1,8	$17{,}2^{a,b}$ +3,9	$15{,}4^{a,b}$ +4,9
	2-nonanol	1101	$37{,}9^{a}$ +8,0	$9{,}3^{a}$ +1,5	$11{,}2^{a}$ +0,2	$23{,}5^{a}$ +6,6	$17{,}6^{a}$ +1,3	$20{,}1^{a}$ +5,7	$18{,}2^{a}$ +1,3	$29{,}2^{a}$ +18,3	$9{,}1^{a}$ +2,5	$25{,}6^{a}$ +2,5	$22{,}2^{a}$ +0,6	$35{,}4^{a}$ +10,3	$36{,}9^{a}$ +13,7
Aldehydes	nonanal	1104	nd	nd	$0{,}7^{a}$ +0,4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

cont. Table 1

1		2	3	4	5	6	7	8	9	10	11	12	13	14	15
Esters	methylbutanoate	747	$11{,}5^{a}$ +3,9	nd	nd	nd	nd	$9{,}2^{a,b}$ +1,6	$2{,}7^{a,b}$ +0,3	$8{,}7^{a,b}$ +7,2	$1{,}6^{a,b}$ +0,5	$5{,}0^{a,b}$ +3,9	$2{,}1^{a,b}$ +1,3	$2{,}17^{a,b}$ +0,77	nd
	ethylbutanoate	802	$33{,}1^{a}$ +6,2	$6{,}5^{a}$ +0,1	$11{,}4^{a}$ +1,4	$15{,}6^{a}$ +1,8	$23{,}4^{a}$ +0,4	$19{,}4^{a}$ +5,9	$17{,}1^{a}$ +4,4	$43{,}5^{a}$ +37,5	$14{,}8^{a}$ +4,6	$25{,}5^{a}$ +0,9	$18{,}9^{a}$ +3,7	$36{,}4^{a}$ +11,9	$36{,}8^{a}$ +2,2
	propylbutanoate	899	$10{,}7^{a,b}$ +4,4	$1{,}8^{b}$ +0,1	$1{,}3^{b}$ +0,1	$7{,}0^{a,b}$ +0,9	$4{,}5^{a,b}$ +0,2	$6{,}4^{a,b}$ +1,6	$9{,}6^{a,b}$ +2,7	$24{,}5^{a}$ +18,2	$8{,}0^{a,b}$ +1,9	$5{,}3^{a,b}$ +0,9	$4{,}4^{a,b}$ +1,0	$8{,}6^{a,b}$ +3,3	$13{,}3^{a,b}$ +0,9
	methylhexanoate	924	$25{,}2^{a}$ +2,9	$0{,}8^{a}$ +0,1	nd	$7{,}8^{a}$ +3,4	$4{,}6^{a}$ +0,9	$28{,}0^{a}$ +13,3	$6{,}1^{a}$ +0,3	$35{,}7^{a}$ +26,6	$5{,}1^{a}$ +2,9	$30{,}5^{a}$ +28,9	$17{,}4^{a}$ +10,5	$11{,}3^{a}$ +10,4	$13{,}7^{a}$ +1,3
	1-methylpropyl-butanoate	996	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	$13{,}0^{a}$ +1,5
	ethylhexanoate	999	$30{,}7^{a}$ +11,3	$13{,}1^{a}$ +0,9	$27{,}2^{a}$ +3,9	$16{,}5^{a}$ +6,0	$27{,}7^{a}$ +3,0	$18{,}5^{a}$ +6,6	$15{,}0^{a}$ +2,2	$52{,}5^{a}$ +35,7	$14{,}7^{a}$ +3,9	$26{,}6^{a}$ +3,5	$21{,}9^{a}$ +2,3	$36{,}2^{a}$ +10,2	$52{,}9^{a}$ +9,9
	1-methylbuthyl-butanoate	1056	$6{,}3^{a}$ +2,3	$0{,}9^{b}$ +0,2	nd	$2{,}2^{a,b}$ +0,7	nd	$2{,}3^{a,b}$ +0,6	$1{,}7^{a,b}$ +0,2	$4{,}9^{a,b}$ +3,3	$1{,}0^{b}$ +0,3	$2{,}2^{a,b}$ +0,4	$2{,}4^{a,b}$ +0,4	$2{,}7^{a,b}$ +1,0	$4{,}7^{a,b}$ +1,6
	propylhexanoate	1094	$13{,}1^{a,b}$ +2,7	$2{,}6^{b}$ +0,6	$3{,}1^{a,b}$ +0,1	$7{,}4^{a,b}$ +2,2	$7{,}2^{a,b}$ +0,4	$5{,}8^{a,b}$ +1,4	$6{,}1^{a,b}$ +0,2	$16{,}1^{a}$ +10,3	$4{,}4^{a,b}$ +1,1	$7{,}8^{a,b}$ +0,3	$6{,}2^{a,b}$ +0,1	$10{,}3^{a,b}$ +2,1	$13{,}5^{a,b}$ +4,8
	methyloctanoate	1123	$3{,}4^{a}$ +0,8	nd	nd	nd	nd	$4{,}2^{a}$ +2,2	nd	$4{,}6^{a}$ +3,2	nd	$4{,}1^{a}$ +3,5	$25{,}0^{a}$ +1,1	nd	nd
	1-methylbuthyl-hexanoate	1137	$3{,}2^{a,b}$ +1,1	$1{,}4^{a,b}$ +0,4	$2{,}7^{a,b}$ +0,1	$3{,}7^{a,b}$ +1,2	$3{,}5^{a,b}$ +0,6	$2{,}8^{a,b}$ +0,8	$4{,}4^{a}$ +0,2	$4{,}6^{a}$ +2,7	$1{,}6^{a,b}$ +0,5	$2{,}0^{a,b}$ +0,2	nd	nd	nd
	ethyloctanoate	1196	nd	nd	$0{,}8^{b,c}$ +0,1	nd	$3{,}9^{a,b,c}$ +0,1	nd	nd	$4{,}3^{a,b}$ +3,0	$1{,}2^{a,b,c}$ +0,2	nd	nd	nd	$5{,}1^{a}$ +2,0
	methyldecanoate	1323	$3{,}2^{a}$ +0,7	nw	nw	$2{,}0^{a}$ +0,8	$3{,}3^{a}$ +0,6	$3{,}9^{a}$ +1,7	$1{,}4^{a}$ +0,1	$6{,}2^{a}$ +3,9	$0{,}9^{a}$ +0,5	$5{,}6^{a}$ +5,5	$3{,}9^{a}$ +3,4	nd	$2{,}8^{a}$ +0,4
	ethyldecanoate	1394	nd	nd	$0{,}9^{b}$ +0,1	nd	$4{,}1^{a}$ +0,5	nd	nd	nd	nd	nd	nd	nd	$5{,}5^{a}$ +2,1

cont. Table 1

1		2	3	4	5	6	7	8	9	10	11	12	13	14	15
Ketones	2-pentanone	728	nd	nd	0,6^{a} +0,3	1,7^{a} +0,3	2,3^{a} +0,3	2,4^{a} +0,2	2,0^{a} +0,6	7,5^{a} +8,7	0,9^{a} +0,1	3,5^{a} +0,5	3,3^{a} +1,8	nd	9,5^{a} +8,5
	3-hydroxy- -2-butanone	739	3,24^{b} +0,5	nd	nd	nd	nd	nd	nd	nd	0,5^{b} +0,01	nd	1,4^{b} +0,2	10,1^{a} +3,1	nd
	2-heptanone	880	86,4^{a} +28,5	13,9^{b} +0,4	10,8^{b} +0,1	19,8^{b} +6,1	11,7^{b} +0,6	24,6^{b} +6,2	18,7^{b} +3,1	54,3^{a,b} +36,6	14,8^{b} +3,9	52,9^{a,b} +4,2	35,7^{a,b} +5,3	45,6^{a,b} +12,3	50,4^{a,b} +3,7
	2-octanone	989	13,6^{a} +3,7	3,7^{b} +0,6	6,3^{b} +1,5	3,1^{b} +0,9	nd	3,2^{b} +0,8	2,1^{b} +0,3	5,6^{b} +3,9	1,5^{b} +0,3	5,0^{b} +1,1	3,6^{b} +0,3	5,7^{b} +1,7	4,7^{b} +0,8
	8-nonen-2-one	1081	18,4^{a} +4,7	4,1^{b} +0,4	3,9^{b} +0,4	6,9^{b} +3,4	4,8^{b} +0,4	5,8^{b} +1,3	5,1^{b} +0,2	12,7^{a,b} +7,2	3,7^{b} +0,7	10,8^{a,b} +0,1	8,7^{a,b} +0,1	10,6^{a,b} +0,2	11,5^{a,b} +1,9
	2-nonanone	1090	98,9^{a} +21,9	18,6^{b} +1,8	19,9^{b} +1,3	33,9^{b} +14,2	29,1^{b} +2,2	33,5^{b} +10,0	26,7^{b} +1,5	72,7^{a,b} +12,2	19,3^{b} +4,6	52,9^{a,b} +0,9	47,7^{a,b} +1,3	63,2^{a,b} +7,6	61,0^{a,b} +17,1
	2-undecanone	1291	9,0^{a} +1,8	2,1^{a} +0,4	3,3^{a} +0,1	3,8^{a} +1,2	6,3^{a} +0,7	4,2^{a} +1,1	3,8^{a} +0,1	9,9^{a} +5,9	2,4^{a} +0,7	4,9^{a} +0,1	5,4^{a} +0,1	6,8^{a} +1,5	8,4^{a} +3,3
Acids	butyric acid	795	59,9^{a} +2,8	14,4^{a} +1,9	18,8^{a} +4,6	29,9^{a} +9,4	33,7^{a} +0,3	25,2^{a} +15,8	23,9^{a} +5,4	58,4^{a} +55,5	19,9^{a} +5,9	41,3^{a} +4,9	36,4^{a} +2,2	nd	60,7^{a} +7,1
	pentanoic acid	810	nd	8,3^{b} +5,7	nd	22,4^{b} +7,5	nd	6,6^{b} +5,8	22,1^{b} +2,7	37,7^{a,b} +15,0	5,7^{b} +0,7	4,5^{b} +1,9	7,9^{b} +6,9	76,5^{a} +33,2	27,2^{b} +9,7
	hexanoic acid	992	nd	10,6^{a,b} +8,1	12,5^{a,b} +0,2	40,2^{a,b} +15,4	45,2^{a,b} +1,1	13,7^{a,b} +4,2	28,9^{a,b} +5,2	51,7^{a,b} +42,8	24,6^{a,b} +5,5	7,2^{a,b} +4,7	11,5^{a,b} +9,1	7,4^{a,b} +2,3	61,6^{a} +14,5
	octanoic acid	1174	12,3^{a,b} +3,6	5,4^{b} +2,1	10,5^{a,b} +1,2	14,3^{a,b} +5,1	27,7^{a} +2,5	6,6^{b} +0,5	9,6^{a,b} +0,5	19,3^{a,b} +14,3	5,8^{b} +1,2	10,3^{a,b} +0,1	9,5^{a,b} +2,3	13,2^{a,b} +2,9	22,3^{a,b} +4,2
	decanoic acid	1364	3,9^{b,c} +1,2	1,2^{b,c} +0,6	2,8^{b,c} +0,5	3,8^{b,c} +1,5	8,9^{a} +1,5	nd	1,7^{b,c} +0,2	4,4^{b} +2,6	1,1^{b,c} +0,1	2,6^{b,c} +0,2	2,1^{b,c} +0,6	nd	4,3^{b,c} +1,1
Hydro- carbons	2,2,4- -trimethylopentane	729	23,0^{a} +5,7	nd	nd	nd	nd	nd	nd	nd	nd	4,6^{b} +5,3	3,9^{b} +4,0	2,8^{b} +1,3	nd
	2,2,4,6,6-pentam- ethyloheptane	988	nd	nd	nd	4,7^{a,b} +2,3	nd	3,1^{a,b} +1,4	2,4^{a,b} +0,6	7,2^{a} +5,2	1,3^{a,b} +0,5	3,8^{a,b} +0,6	3,9^{a,b} +0,1	5,4^{a,b} +0,8	4,6^{a,b} +0,7

Explanatory notes: RI – retention index; nd – not detected; Table shows mean values and standard deviation; n = 3; a–d – mean values determined in lines and denoted by different letters differ statistically significantly at $p < 0.05$

Noticeable is the high turnover that of most classes of compounds in the fragrance profile of the samples stored at 25°C. After an initial sharp decline of the shares of most classes of compounds in the first day of storage, there was also a sharp increase in their participation at day third and another sharp decline in the fourth day (Figure 2).

The Rokpol cheese samples stored for one day at 35°C were characterized by a decrease in the intensity of most groups of compounds, as in the case of other storage conditions, but the level of decrease was smaller. In the following days there has been a regular increase in the proportion primarily of acids, esters and ketones in the general volatile profile (Figure 3).

Esters and ketones had the highest share in the volatiles profile of all the group of compounds in all samples after four days of storage at ambient conditions and elevated temperature, and after four weeks of storage in refrigerator with the predominant compounds: 2-nonanone and 2-heptanone, which is confirmed in studies of other scientists (MOLIMARD and SPINNLER 1996).

Both aldehydes and hydrocarbons had the smallest share in the volatile fraction of Rokpol cheese regardless of the conditions and storage time and similar results were noted by YVON and RIJNEN L 2001. Already in the first tested samples significantly decreased of the share of hydrocarbons and in the later stages remained without statistically significant changes. In contrast, nonanal as the only compound belonging to the aldehydes was detected in only one of all the tested samples (Table. 1).

Principal Components Analysis

Appointed 12 principal components, of which the first four explaining total of 83.72% of the variation were chosen to describe the phenomenon.

Table 2

Percent rate of total variability explained by principal components obtained in PCA analysis

Principal component	% of total variation	Cumulative % of variability
1	42.13	42.13
2	18.51	60.64
3	12.35	72.99
4	10.73	83.72
5–12	16.28	100

The first principal component explained 42.13% of the total variability. It showed a strong negative correlation (0.895 – 0.974) from 2-pentanol, propyl ester, hexanoic acid, 2-heptanone, 2-nonanone, 2-nonanol, 2-undecanone and 8-nonene-2-one.

The second principal component explained 18.51% of the total variability. The same as the first principal component it clearly differentiated control sample form those which were stored. Particularly large differentiation related to the control and cheese samples stored for four days in the incubator and 4 weeks in the refrigerator (Figure 1). The stored cheese samples had larger share of hexanoic acid, decanoic acid ethyl ester and octanoic acid. In contrast, the control sample had the most of 2-oktanone and 2-butanoic acid methyl ester.

The third principal component explained 12.35% of the total variability and significantly separated the samples taken at day three of storage under 25°C and 35°C (Figure 2). Differentiation of the samples was based on decanoic and octanoic acid methyl esters and and 1,3-propanediol, which high content were determined in a sample at 25°C. In contrast, the sample taken at 35°C distinguished by a high content of 3-hydroxy-2-butanone, and 2,3-butanediol.

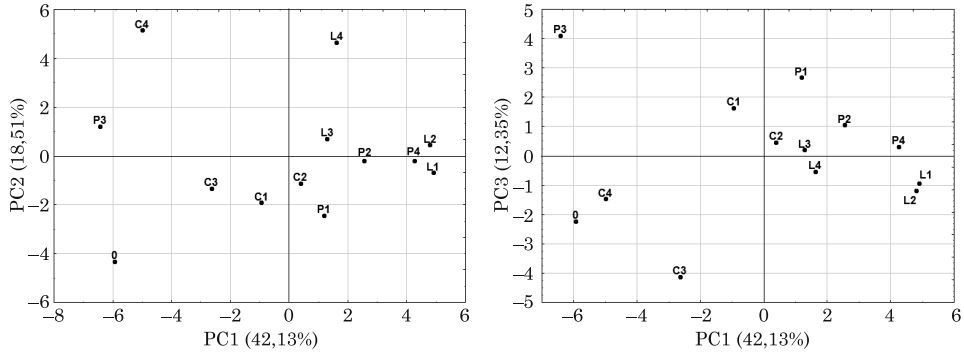

Fig. 4 and 5. PCA analysis results – projection of cases: PC1/PC2 (1), PC1/PC3 (2)

The fourth principal component explained 10.73% of the total variability and, as in the case of the third main component, the sample taken after three days of storage at 35°C was clearly separated from the other (Figure 6). The effect on the differentiation had a high pentanoic acid and 1,3-propanediol in the mentioned sample. The compounds were highly and moderately positively correlated (0.836 and 0.684 respectively) with the discussed main component. In contrast, the separation control sample from other samples was caused by decanoic acid and butanoic acid, and 2,2,4-trimethylpentane. The fourth principal component of showed a moderate negative correlation with these compounds (0.511 – 0.560).

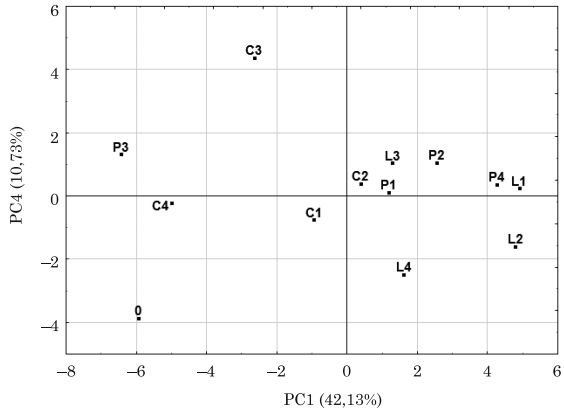

Fig. 6. PCA analysis results – projection of cases: PC1/PC4

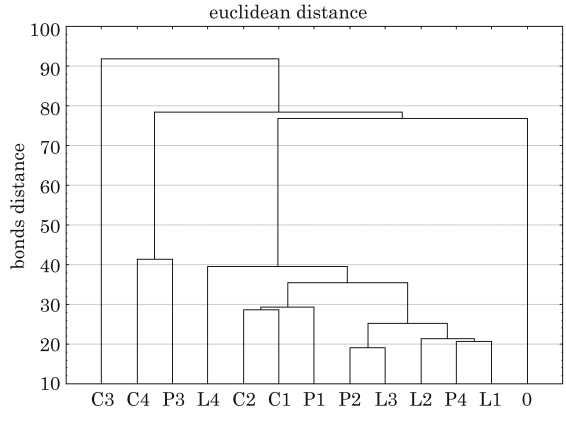

Fig. 7. Results of cluster analysis based on volatile profiles of investigated cheese samples – Ward's method (euclidean distance)

Cluster Analysis

Cluster analysis allowed to group Rokpol cheese stored samples with similarities in the composition of the volatile fractions. The similarities between established groups were reflected in the PCA test presented earlier. The dendrogram shows four groups of tested samples and three samples not classified to any group. The first two groups of samples were stored in the refrigerator for 3 weeks and samples from the second and fourth day of storage at 25°C (Figure 7). Another group of samples were stored for one day at 25°C and samples from the first and second day of storage at elevated temperature. The last group formed flavoring profiles of samples from the third day of

storage at 25°C and the fourth day at the elevated temperature. The control sample and the one from the third day of storage at elevated temperature differed from others mostly. The profile of volatile compounds of the sample from the fourth week of refrigerated storage also significantly differed. However, the chemical composition of the volatile fraction showed an affinity of that sample with the three previously described groups.

Conclusions

1. Based on cluster analysis it is visible that cheese storage for one week in the refrigerator or two days in 25°C has the same sensory effect. This is an evidence for the fact that refrigerator prolongs aroma stability.

2. The storage of cheese at 25°C and 35°C gives similar aroma result.

3. The analysis of volatile compounds profiles in different temperature conditions of cheese storage show the direction and intensity of sensory changes. It also allows for evaluation of fragrance stability and for selection proper storage parameters.

4. Obtained results show statistically insignificant slight qualitative differences in volatile fractions of tested cheese.

5. Application of headspace solid-phase microextraction (HS-SPME) in connection with statistic tools (PCA, CA) can be an useful tool in analysis of volatile compound profiles in cheese, as well as study changes of these profiles during storage or ripening.

Translated by Authors

Accepted for print 7.12.2015

References

Bzducha A., Obiedziński M. W. 2006. Badania nad związkami lotnymi serów z przerostem pleśni techniką analizy fazy nadpowierzchniowej (SPME) i chromatografii gazowej sprzężonej ze spektrometrią mas (GC/MS). Żywność. Nauka. Technologia. Jakość, 47(2): 36–47.

Curioni P.M.G., Bosset J.O. 2002. Key odorants in various cheese types as determined by gas chromatography-olfactometry. Int. Dairy J., 12(12): 959–984.

Fox P.F., McSweeney P.L.H. 2004. Cheese. Chemistry, Physics and Microbiology Cheese: An Overview. Elsevier Applied Science, Amsterdam.

Fox P.F., Singh T.K., McSweeney P.L.H. 1995. Chemistry of structure – function relationship in cheese. Biogenesis of flavour compounds in cheese. Springer Science+Business Media, New York.

Frank D.C., Owen C.M., Patterson J. 2004. Solid phase microextraction (SPME) combined with gas-chromatography and olfactometry-mass spectrometry for characterization of cheese aroma compounds. LWT – Food Sci. Technol., 37(2): 139–154.

Guinee T.P., McSweeney P.L.H. 2006. Advanced Dairy Chemistry Volume 2 Lipids. Significance of Milk Fat in Cheese. Springer Science Business Media, New York.

Jeleń H.H. 2004. Związki zapachowe żywności wyzwanie dla analityka. Przemysł Spożywczy, 5: 18–24, 47.

Kovats Retention Index. [online 05.05.2015]: http://www.pherobase.com/database/kovats/kovats-index.php.

MARILLEY L., CASEY M.G. 2004. Flavours of cheese products: metabolic pathways, analytical tools and identification of producing strains. Int. J. Food Microbiol., 90(2): 139–159.

MOLIMARD P., SPINNLER H.E. 1996. Compounds Involved in the Flavor of Surface Mold-Ripened Cheeses: Origins and Properties. J. Dairy Sci., 79(2): 169–184.

SURBURG H., JOHANNES P. 2006. Common Fragrance and Flavor Materials. 5. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

TRIHAAS J., VOGNSEN L., NIELSEN P.V. 2005. Electronic nose: New tool in modelling the ripening of Danish blue cheese. Int. Dairy J., 15(6-9): 679–691.

VITOVA E., LOUPANCOVA B., ZEMANOVA J., ŠTOUDKOVA H., BREZINA P., BABAK L. 2006. Solidphase microextraction for analysis of mould cheese aroma. Czech J. Food Sci., 24(6): 268–274.

Yvon M., Rijnen L. 2001. Cheese flavour formation by amino acid catabolism. Int. Dairy J., 11(4–7): 185–201.