Food Chemistry 111 (2008) 988–997

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Composition of volatile compounds of honey of various floral origin and beebread collected in Lithuania

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ARTICLE INFO

Article history:
Received 13 November 2007
Received in revised form 1 April 2008
Accepted 2 May 2008

Keywords:
Honey
Beebread
Volatile compounds
SPME–GC/MS

ABSTRACT

Honey is collected from various flowering plants and its composition, particularly volatile flavour compounds to some extent depends on the nectar source. Therefore, some volatile constituents may be indicators of honey origin. In this study the volatile profiles of 15 honey samples of different botanical origin and one beebread sample are characterised. Volatiles were collected by means of SPME and analysed by GC/MS. Botanical source of honey samples was established by the melissopalynological method: 11 of analysed samples were unifloral rape honeys, 1 clover, 1 caraway and 2 polyfloral. In total 93 compounds in honey and 32 in beebread were identified. They involve different classes of chemical compounds, including alcohols, ketones, aldehydes, acids, terpenes, hydrocarbons, benzene, and furan derivatives. Benzaldehyde and benzenacetaldehyde were the only compounds found in all 15 honey sample. Dimethyl sulphide, pentanenitrile, benzylnitrile were identified in 14 honeys; isobutane, octanoic and nonanoic acids in 13 samples; furfural, linalool and nonanal in 12 samples; octanal, lilac aldehyde C, hotrienol and decanal in 11 samples and finally 2-methylbutanenitrile in 10 honey volatile fractions. Remarkable variations were observed in the composition of volatiles in honey from different sources. In addition, volatile profiles of honey samples were analysed after 3 months of storage and it was found that the amount of headspace volatiles in the majority of samples decreased.

1. Introduction

Honey is a sweet and flavourful product, produced by bees from nectar and/or honeydew; it has been consumed since the ancient times as a high nutritive value food distinguished by its characteristic aroma and pleasant sweet taste. In general, aroma of honey is formed by volatile compounds, which may come from the nectar or honeydew collected by bees; consequently it may largely depend on the plant of honey origin. Additionally, flavour constituents may be formed by the honeybee, as well as during thermal processing and/or storage of honey (Bonvehí & Coll, 2003; Soria, Martínez-Castro, & Sanz, 2003).

More than 400 different compounds have been identified in the volatile flavour fraction of honey originated from different floral types (Bentivenga, D'Auria, Fedeli, Mauriello, & Racioppi, 2004). Taking into account that aroma composition of some types of honey has not been yet studied and the sensitivity of analysis continuously improves, it is likely that the number of identified honey volatiles will further increase. Typical volatile components can be identified for honeys from some definite floral sources; such compounds are specified as floral markers of the corresponding honey. Some compounds were reported as characteristic components of honey from certain geographic regions. For instance, it was suggested that English honeys can be identified by the presence of 1-penten-3-ol as a specific compound for this region (Radovic et al., 2001).

Some types of honey can be distinguished by one characteristic compound; however the aroma of the majority of honeys depends on the group of constituents. For instance, furfuryl mercaptan, benzyl alcohol, δ-octalactone, γ-decalactone, eugenol, benzoic acid, isovaleric acid, phenylethyl alcohol, and 2-methoxyphenol were reported to be particularly important impact volatile compounds for Brazilian caju honey (Moreira, Trugo, Pietroluongo, & De Maria, 2002). It is worth mentioning that phenylethyl alcohol is well known in perfume industry as possessing floral, spicy, and herb-like odour. This compound was reported as an important aroma compound in lime honey (Moreira et al., 2002). However, Radovic et al. (2001) found phenylethyl alcohol only in two lime honey samples of the four analysed and concluded that the authenticity of such honeys may be confirmed by the presence of one of the following substances: 2-methylfuran, α-terpinene, α-pinene oxide, bicyclo-3,2,1-octane-2,3 bis (methylene), methyl isopropyl benzene, aromatic hydrocarbon, 3-cyclohexen-1-ol-5-methylene-6-isopropylene, 4-methylacetophenone.

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doi:10.1016/j.foodchem.2008.05.021

Although the variability of honey flavour depends mainly on its floral origin, the isolation and detection techniques of volatiles may also play an important role in analysis results (Alissandrakis, Tarantilis, Harizanis, & Polissiou, 2005; Anklam, 1998). There are many isolation techniques of volatile components which may be applied to honey. The concentration of volatile compounds in honey is very low, therefore before their isolation it is highly recommended to remove sugars, which are the major honey components. Various isolation methods have been used so far, e.g. Likens–Nickerson simultaneous steam distillation extraction (Bouseta & Collin, 1995), dynamic headspace extraction (Bianchi, Careri, & Musci, 2005; Radovic et al., 2001), ultrasound-assisted extraction (Alissandrakis et al., 2005), hydrodistillation (Alissandrakis et al., 2005), solvent extraction (Bonvehí & Coll, 2003), solid phase microextraction (Bentivenga, D'Auria, Fedeli, Mauriello, & Racioppi, 2004). All isolation techniques have specific advantages and disadvantages. Some of them are time-consuming (e.g., hydrodistillation), particularly when a large number of samples are analysed; the use of solvent is associated with the loss of volatiles during solvent removal; while heating may result in the formation of artefacts, particularly due to the thermal degradation of sugars (Alissandrakis et al., 2005). Various modifications of headspace can overcome some of the above mentioned disadvantages; in addition, the profiles of headspace volatiles are more closely associated with sensory perceptions.

The main task of our research is to comprehensively characterise Lithuanian honeys obtained from various sources. Recently, we reported antimicrobial and antioxidant properties of Lithuanian honey samples (Baltrušaitytė, Venskutonis, & Čeksterytė, 2007a, 2007b), while the volatile compounds of honey and beebread from Lithuania were not analysed until now. The aim of this study was to assess the composition of volatile aroma compounds and their changes during storage in various honey and beebread samples collected in Lithuania. Solid phase microextraction (SPME), a rapid, solvent-free, and inexpensive technique was selected for the collection of volatiles from honey and beebread.

2. Material and methods

2.1. Honey samples and their classification

Honey samples were obtained from apiarists throughout Lithuania, mainly from the central part of the country (Kedainiai district), two samples were from Radviliskis and Salcininkai districts. All honey and beebread samples were collected during the flowering season, from May to August, 2005. Between 20 and 25 bee families are settled in a several locations (Akademija, Baisogala, Degesiai, Jasiunai, Girine, Krakes, Lazai, Paberze, Spitolpievis, Slapaberze). All bee families are settled in a distance exceeding 5 km from each other in the rural area or near the forest. Usually, the bees from the same site collect the nectar approximately 5 km around their location, and as a rule from the same plant species. On average 500–600 kg of honey was collected in each region from 20 to 25 bee families (25–30 kg honey/per bee family). Honey was extracted to the 50-kg containers (approximately 10–13 containers from each location). Then five representative samples were collected from the container for the analyses by placing them in 0.5 kg jars and sealing hermetically. The samples were stored in a dry and cool place (6–8 °C temperature) before the analysis. First, a series of analysis was performed four month after collection and this honey is further referred to as 'fresh honey'.

The floral source of honey samples was determined by the melissopalynological method (Louveaux, Maurizzio, & Vorwohl, 1978; Persano Oddo, Piazza, Sabatini, & Accorti, 1995), which is based on the relative frequency of the pollen from nectar secretion plants. Different opinions exist regarding the use of pollen present in the honey for the indication of its botanical origin (Molan, 1998); however until the present date this method has been frequently used for this purpose. Pollen species were identified by using previously published data (Burmistrov & Nikitina, 1990; Straka, 1975) and pollen collection of well-known plants, which was prepared for microscopy at the Apicultural Department of the Lithuanian Institute of Agriculture. The prepared slides were examined using microscope (×400) for the identification of pollen in honey and counting honeydew elements. At least 500 pollen grains (PG) and honeydew elements (HDE) were counted in 100 microscopy fields. All plant elements were observed separately. After the identification of PG and HDE in the given samples, the pollen of nectarless plants and the HDE were deducted from the sum total. The content of the pollen of nectar plants to botanical composition of honey was calculated and expressed in percentages. HDE were calculated as a percentage of sum total PG and HDE according to the formula: HDE = HDE/(PG + HDE)∗100 (%).

The following honey types were classified (Table 1): unifloral from winter and spring rape (WR2, WR3 and SR6-SR14), polyfloral spring and summer (POL1, POL15), unifloral summer from caraway (CAR4) and white clover (WCL5). Unifloral honey met the botanical and chemical composition requirements established by the rules of the International Commission for Bee Botany, presently called International Commission for Plant–Bee Relationships (Louveaux et al., 1978) and monograph of methods and standards (Persano Oddo et al., 2004; Von der Ohe, Persano Oddo, Piana, Morlot, & Martin, 2004). It should be noted that the main honey plant in Lithuania is spring rape (*Brassica napus* L. ssp. *oleifera annua* Metzg.) and in honey from those plants spring rape pollen were over-represented.

2.2. SPME and GC–MS analysis

Extraction of honey and beebread volatiles was performed on CAR/PDMS (Carboxen-Polydimethylsiloxane fibre (Supelco, Bellefonte, PA). Before analysis, the fibre was preconditioned in the injection port of a gas chromatograph according to the instruction provided by the manufacturer.

Sample preparation was carried out by mixing 30 g of honey or beebread and 10 mL of saturated NaCl solution (in order to establish equilibrium) in 100 mL vials with PTFE/silicone septa and a stirring bar. The samples were kept and magnetically stirred for 20 min at 40 °C to allow equilibrium. Sampling of the volatile honey compounds was performed by inserting the sheathed fibre through the septum and exposing it to headspace for 30 min. The fibre was then retracted and transferred to the injector port of the chromatograph where the compounds were desorbed for 3 min.

Analysis of volatile compounds was carried out using *Perkin Elmer Clarus 500* gas chromatograph equipped with a *Perkin Elmer Clarus 500* series mass spectrometric detector (Perkin Elmer Instruments, Shelton, USA) in the electron impact ionisation mode at 70 eV, the mass range was *m/z* 29–550. Volatile compounds were separated using an Elite–5 MS capillary column (dimethylpolysiloxane, 5% diphenyl, 30 m length, 0.25 mm i.d., 0.25 μm film thickness, Perkin Elmer Instruments, Shelton, USA). The oven temperature was programmed as described above. Carrier gas, helium was adjusted to a linear velocity of 36 cm/s at 50 °C or 1.0 mL/min volumetric flow. Split mode was used at a ratio of 1:20; injector temperature was 250 °C. The oven temperature was maintained at 40 °C for 5 min, then raised 5 °C/ min to 250 °C and held 10 min. Three replicates of each sample were run three times by GC–MS.

The identification of the isolated volatile compounds was performed by their Kovats retention indices (KI) and mass spectra (NIST vers. 1.7 and literature data). KIs were determined by using

Table 1
Melissopalynological analysis of tested honeys samples

Sample code	Collection location/ date	Botanical origin of pollen, %
POL1	Slapaberze 2005 05 31	Winter rape (*Brassica* napus var. *oleif. F. biennis Thellung*) – 33.6; willow (*Salix alba L., Salix caprea L.*) – 18.2; dandelion (*Taraxacum officinale L.*) – 20.2; apple (*Malus domestica Borkh.*) – 18.5; white clover (*Trifolium repens* L.) – 4.9; field scabious (Knautia arvensis L.Coult.) – 4.6
WR2	Akademija 2005 06 04	Winter rape – 68.9; dandelion – 15.0; willow -13.2; apple – 2.9
WR3	Akademija 2005 06 05	Winter rape – 87.0; willow – 5.9; dandelion – 3.8; apple – 3.3
CAR4	Baisogala 2005 06 29	Caraway (*Carum carvi*. L.) – 53.0; raspberry (*Rubus idaeus* L.) – 15.9; spring rape (*Brassica napus* L. *ssp. oleifera annua* Metzg) – 15.9; charlock (*Sinapis* L.) – 6.9; white clover – 8.3, honeydew – 4.6
WCL5	Jasiunai 2005 07	White clover – 47.7; raspberry – 29.2; apple – 12.7; spring rape – 5.6; caraway – 4.8
SR6	Lazai 2005 07	Spring rape – 68.4; white clover – 9.5; raspberry – 5.4; cornflower (*Centaurea cyanus* L.) – 5.4; caraway – 4.9; salvia (*Salvia* L.) – 4.0; chicory (*Cichorium* L.) – 2.4
SR7	Degesiai 2005 07 22	Spring rape – 64.1; caraway – 15.8; cornflower – 7.6; salvia – 6.0; white clover – 4.5; thistle (*Cirsium* L.) – 2.0
SR8	Girine 2005 07 25	Spring rape – 62.7; red clover (*Trifolium pratense* L.) – 14.0; white clover – 8.7; cornflower – 9.3; caraway – 5.3
SR9	Paberze-Uzupe 2005 07 26	Spring rape – 61.8; willow – 13.8; cornflower – 13.2; dandelion – 4.6; caraway -3.3; blackberry (*Rubus caesius* L.) – 3.3;
SR10	Spitolpievis 2005 07 28	Spring rape – 50.6; red clover – 38.3; white clover – 6.2; buckwheat (*Fagopyrum* Gaertn.) – 4.9; honeydew – 23.8
SR11	Krakes 2005 07 28	Spring rape – 48.8; red clover – 35.0; white clover – 11.0; charlock -5.2
SR12	Slapaberze 2005 07 26	Spring rape – 92.6; charlock – 4.3; cornflower – 3.1
SR13	Uzupe 2005 08	Spring rape – 69.6; white clover – 9.0; red clover – 5.3; cornflower – 13.1; caraway – 3.0; honeydew – 5.5
SR14	Degesiai 2005 08 25	Spring rape – 67.5; cornflower – 11.9; caraway – 8.1; white clover – 7.2; chicory – 1.9; buckwheat – 3.4; honeydew – 14.7
POL15	Spitolpievis 2005 08 26	Spring rape – 35.8; red clover – 35.2; white clover – 9.5; buckwheat – 15.0; chicory – 4.5; honeydew – 24.1
BB	2005	Mixture of honey and beebread: spring rape – 35.9; white clover – 15.8; bluebottle (*Centaurea cyanus* L.) – 12.2; willow – 10.1; lime (*Tilia* L.) – 7.9; charlock – 7.9; caraway – 5.8; alder (*Frangula* L.) – 2.9; white clover – 1.4

POL – polyfloral honey, WR – winter rape honey, SR – spring rape honey, CAR – caraway honey, WCL – white clover honey, BB – beebread.

homologous series of normal *n*-alkanes, C_8–$C_{30,32}$ (Sigma Chemical Co., St. Louis, MO) in a temperature-programmed GC run, as described above (IUPAC., 1997). Positive identification was assumed when good matches of mass spectra and KI were achieved. The data obtained was also compared with various literature sources (Adams, 2001; Alissandrakis, Tarantalis, Harizanis, & Polissiou, 2007; Bentivenga, D'Auria, Fedeli, Mauriello, & Racioppi, 2004; De la Fuente, Martínez-Castro, & Sanz, 2005; De la Fuente, Sanz, Martínez-Castro, Sanz, & Ruiz-Matute, 2007; Lusic, Koprivnjak, Curic, Sabatini, & Conte, 2007; Piasenzotto, Gracco, & Conte, 2003; Soria et al., 2003).

The amount was assessed by the total peak ion current areas. Relative area values (percentage of total volatile composition) were used for quantification purposes. In order to determine the variation of volatile compounds of the honey, the same honey samples were analysed after three months storage in the dark at room temperature. The changes of volatiles were assessed by the comparison of their percentages in the aroma concentrates.

2.3. Statistical analysis

The results are provided as a mean of three measurements. Standard deviations (SD) were in the range of 3–10%, with a few exceptions. Standard deviations were calculated using spreadsheet software (Excel®). To determine whether differences among averages were significant, single-factor ANOVA was applied (Excel®).

3. Results and discussion

3.1. Volatile compounds of honey

The profiles of volatile honey fractions were very complex; about 100 compounds were detected in the SPME extracts isolated from the 15 samples of different botanical origin by GC/MS. The identified components involve different classes of chemical compounds including alcohols, ketones, aldehydes, acids, terpenes, linear and branched hydrocarbons, benzene, and furan derivatives (Table 2). However, the chromatographic profiles of the majority of the analysed honey samples were quite similar in terms of their qualitative composition; while the intensity of some GC peaks varied in a wide range. Slightly different GC profiles were recorded for CAR4, WCL5, SR12 and POL15 honey. Typical chromatograms of rape origin honey samples are presented in Fig. 1.

Benzaldehyde and benzenacetaldehyde were the only compounds found in all 15 honey samples. Some other components were also common for various honey samples. Thus, dimethyl sulphide, pentanenitrile, benzylnitrile were identified in 14 honeys; isobutane, octanoic and nonanoic acids in 13 samples; furfural, linalool and nonanal in 12 samples; octanal, lilac aldehyde C, hotrienol and decanal in 11 samples and finally 2-methylbutanenitrile in 10 honey volatile fractions. Other volatile compounds were found in fewer honey samples (Table 2).

To the best of our knowledge, several compounds have not been previously reported to be present in honey. They include isobutane, 3-methylhexane, 1,4-pentanediol, 2,3-dihydroxypropanal, 4-methyloctane, 3-caren-2-ol, 2,3-dimethylheptane, 4-methyl-2,7-octadiene, *trans*-sabinene hydrate, *p-sec*-butyltoluene, *p*-ethylcumene, 5,9-dimethyl-1-decanol.

It is well established that aroma of bee honey is highly dependent on volatile fraction composition, which in turn depends on nectar composition and floral origin (Cuevas-Glory, Pino, Santiago, & Sauri-Duch, 2007). Our results to some extent are in agreement with these findings. The highest variety of volatile compounds was determined in unifloral caraway (CAR4) and rape honeys (SR12, SR14) and polyfloral honey (POL15); volatile profiles from these samples consisted of 40 compounds. Carvacrol and *p*-cymene are very abundant in various plants and were attributed as markers for lime tree honey (Lusic et al., 2007); we found these compounds in some samples of spring rape honey as well. It should be noted that there was a small amount of lime tree pollen in the rape honey sample analysed in our study; however, following the existing recommendations lime tree pollen were not included in the composition due to a content lesser that 1%. The samples containing important compounds for lime tree honey were harvested at the end of June or July, i.e. at the beginning or at the end of lime tree

Table 2
The percentage composition of identified volatile compounds in the honey samples (fresh honey/after three months of storage),%

No.	KI[a]	Compound	POL1	WR2	WR3	CAR4	WCL5	SR6	SR7	SR8	SR9	SR10	SR11	SR12	SR13	SR14	POL15	Identification
1	ND	Ethanol	1.7/2.1	0.3/0.3	0.8/1.6	2.0/3.1		4.1/20.2					0.7/0.7	0.3/0.3	7.2/6.6	1.6/1.2	0.2/0.7	MS, L
2	ND	Isobutane		5.9/4.0	8.7/4.2	4.7/4.4	1.1/7.6	6.9/9.1	2.6/7.1	1.0/7.6	3.6/6.1	5.5/7.6	10.9/9.5		4.0/3.0	4.6/4.5	1.8/2.5	MS
3	ND	Dimethyl sulphide	0.0/0.8	5.0/3.4	2.1/3.3	7.4/4.5	3.2/1.5	15.4/16.2	4.1/10.7	6.3/9.5	15.5/12.3	8.6/11.5	7.9/13.0	8.5/12.2	10.2/10.9	11.6/12.7	1.4/1.4	MS, L
4	ND	Acetic formic anhydride		0.8/1.7		2.3/0.7	1.7/0.9	3.7/3.5	0.1/0.4		14.8/12.5	3.2/1.2			2.7/1.8			MS, L
5	ND	Hexane			3.3/0.0			0.9/0.0				3.7/2.7	4.0/0.0		1.6/3.4	1.6/1.3	8.1/2.0	MS, L
6	ND	Ethyl acetate	13.6/0.9	0.6/1.8	3.2/1.2		0.5/1.1	2.2/5.0	0.5/1.5	2.5/1.4	1.4/4.7	2.0/1.2		1.0/0.4	1.3/1.3			MS, L
7	ND	Chloroform							0.2/0.5	2.8/2.3								MS, L
8	ND	2-Methylpropanenitrile	0.0/6.5	12.7/0.5	14.7/12.1	0.4/1.3	8.6/6.0		0.6/2.7			2.5/2.5			1.3/1.3	0.6/0.5	0.3/1.4	MS, L
9	ND	3-Methylbutanal				1.9/0.9			1.7/0.5		0.7/1.0	0.8/0.0		0.2/0.2	0.7/1.1	0.6/0.5	4.7/7.4	MS, L
10	ND	3-Methylhexane											7.7/0.2				2.2/3.1	MS
11	ND	Pentanal				1.8/1.6												MS, L
12	ND	1,4-Pentanediol				0.4/0.2												MS
13	ND	Heptane															0.4/0.5	MS, L
14	ND	2-Methylbutanenitrile	1.1/7.8	7.3/9.7	12.1/10.1		12.4/10.3		6.6/4.8	1.2/1.2		2.7/3.5	0.0/0.9		1.3/1.3	2.3/2.1	0.9/1.4	MS, L
15	ND	Pentanenitrile	5.4/3.9	11.2/20.4	8.5/7.7	7.8/7.8	4.4/8.2	5.8/1.9	3.7/6.6	10.2/8.1	4.8/2.3	11.9/10.3	4.9/5.7		7.4/6.8	8.3/8.7	4.0/6.4	MS, L
16	ND	1-Pentanol															0.7/0.9	MS, L
17	ND	Dimethyl disulphide						1.4/0.9		0.7/0.6	0.5/1.1		0.0/2.1	0.3/0.3	2.0/1.4	2.5/2.2		MS, L
18	ND	2,3-Dihydro-5-methylfuran				2.1/1.0												MS, L
19	ND	2-Methyl propanoic acid		3.5/0.0		1.4/0.8	7.7/3.0											MS, L
20	ND	Isobutylbenzene			2.4/3.0	0.5/0.6	1.4/0.0		2.7/1.7	0.9/0.8								MS, L
21	ND	Toluene										1.3/1.5	2.5/0.5	0.1/0.2		1.2/1.1	7.6/2.1	MS, L
22	ND	Butanoic acid															0.5/1.4	MS, L
23	ND	4-Pentenal				1.4/0.8												MS, L
24	802	Octane				0.2/6.1					1.9/4.9	0.6/0.6	1.6/3.1				0.7/1.1	MS, L, KI
25	803	2,4-Dimethylheptane		1.9/0.0				1.4/4.0	2.2/3.2	6.6/6.6				1.2/0.8	2.9/2.9	4.3/3.5		MS, L
26	805	1-Octene							0.0/0.1									MS, KI
27	808	2-Octene				1.6/0.9												MS, L, KI
28	810	Hexanal												0.0/0.5				MS, L, KI
29	840	2,3-Dihydroxypropanal		0.0/1.9		0.2/0.0										1.8/1.5		MS
30	844	Furfural				2.7/2.4	3.4/2.5	3.9/2.1	7.3/4.3	8.2/6.5	6.2/6.5	9.5/8.1	9.3/7.6	1.2/1.3	6.1/5.3	0.8/7.3	25.5/22.8	MS, L, KI
31	852	2-Hepten-1ol								10.9/9.3		10.5/7.5		0.0/0.1	7.5/1.3	12.6/11.7	3.4/5.7	MS, L
32	853	Hexanenitrile			1.0/2.2	5.6/4.5		2.4/1.8										MS, L
33	855	4-Methylpentanenitrile	16.4/21.7		6.9/4.0		1.6/5.5		5.4/1.4	1.9/1.9	4.9/5.5							MS, L
34	859	4-Butoxy-1-butene		2.0/0.0														MS, L
35	867	3-Methyl butanoic acid	3.9/4.6	11.5/4.9		1.1/1.4	4.8/7.9		6.6/3.4		1.0/0.7	9.2/1.8				0.9/1.0	4.6/4.7	MS, L, KI
36	869	4-Methyloctane												0.0/0.1				MS
37	871	Pentanoic acid															5.6/2.7	MS, L
38	872	2-Methyl butanoic acid	13.7/6.5						0.5/0.0									MS, L
39	900	Nonane		0.7/4.9		4.5/0.2			0.0/0.6	0.8/0.7					0.9/0.9	3.2/3.1		MS, L, KI
40	906	Heptanal				1.2/1.6				1.7/1.5	3.9/2.6		2.7/0.9	0.6/0.7	2.6/1.2	0.4/0.3	1.3/0.0	MS, L, KI
41	911	2-Methyl-1-pentanol															0.7/0.4	MS, L
42	928	Cumene												0.1/0.2				MS, L, KI
43	958	3-Methyl pentanoic acid	2.4/1.4	2.0/2.2		1.6/0.8	3.4/3.5	0.9/0.0	3.3/2.4	1.0/1.0	0.0/0.6	0.0/0.4	0.0/1.0			1.4/1.3	0.3/0.0	MS, L, KI
44	969	Benzaldehyde	3.3/4.5	1.8/2.8	9.5/6.1	21.4/20.4	7.0/6.2	7.7/5.1	6.6/8.8	8.6/7.1	5.7/3.0	5.2/5.9	5.8/8.6	2.3/2.1	1.1/2.2	3.6/3.2	7.3/10.1	MS, L, KI
45	973	Dimethyl trisulphide						0.0/0.5		1.3/1.2	1.1/0.9				2.2/2.6	1.2/1.1		MS, L
46	990	Hexanoic acid				tr./1.4	1.2/1.8	2.3/1.3	0.0/0.7	1.3/0.7	2.1/1.3	1.4/1.2	0.0/0.8	0.0/0.1	0.0/0.9	1.2/1.1	1.0/0.5	MS, L, KI
47	999	Decane				0.7/0.5												MS, L, KI

48	1006	3-Caren-2-ol											0.6/0.7				MS
49	1007	Octanal				0.1/1.8	0.7/1.2	0.9/1.0	1.2/0.9	1.3/1.2	1.0/0.9	0.5/0.4	0.0/0.5	6.0/1.2	1.3/1.7	3.8/2.9	0.4/0.7	MS, L, KI
50	1011	2,3-Dimethylheptane				0.3/0.2												MS
51	1012	cis-Dehydroxy linalool oxide						0.5/0.7	1.0/0.8		0.7/0.4	0.5/0.4		3.0/3.5				MS, L, KI
52	1019	2-Carene												0.0/0.5				MS, L
53	1027	*p*-Cymene												2.3/2.6	2.0/0.8	0.4/0.4		MS, L, KI
54	1031	Limonene				0.1/0.1												MS, L, KI
55	1033	β-Phellandrene												0.3/0.3				MS, L, KI
56	1043	Benzyl alcohol				tr./0.5		1.9/0.0		0.9/0.8	1.0/0.6	0.7/1.2	1.2/1.8	1.0/1.0	0.0/0.9	1.0/0.8		MS, L, KI
57	1052	Benzenacetaldehyde	15.2/11.2	7.1/6.2	14.3/10.9	2.1/2.7	5.9/4.1	2.6/1.1	3.5/2.3	2.3/2.5	4.2/2.2	1.0/1.8	2.2/2.4	0.4/0.4	1.7/1.1	1.3/1.1	0.8/1.3	MS, L, KI
58	1076	trans-Linalool oxide				0.2/0.1				0.4/0.4							0.3/0.3	MS, L, KI
59	1079	Heptanoic acid				0.6/0.7		0.0/0.5			0.3/1.0			0.0/0.5		0.4/0.3		MS, L, KI
60	1081	*p*-Cresol															0.6/0.7	MS, L, KI
61	1093	*p*-Cymenene				0.0/0.6		2.8/2.8	1.5/2.0	1.3/1.1	1.2/1.3	1.2/2.9	0.0/1.3	37.9/40.6	12.3/8.3	1.9/1.8	0.5/0.4	MS, L, KI
62	1096	2-Nonanone															0.3/0.0	MS, L, KI
63	1099	Undecane				0.7/0.1	0.8/1.3	0.7/0.0				0.6/0.0				0.3/0.3		MS, L, KI
64	1102	Linalool				0.8/1.3	0.8/1.4	0.9/0.8	1.3/1.1	1.3/1.2	0.5/0.7	0.6/1.1	1.5/2.5	0.2/0.3	0.9/1.2	0.7/0.6	0.3/0.4	MS, L, KI
65	1106	Hotrienol		0.7/1.5	0.0/1.5	0.0/0.1	0.2/0.7	3.3/2.8	5.4/5.9	0.0/4.8	4.4/3.8	3.1/4.2	3.0/4.9	1.9/3.1	3.2/4.6	3.6/3.2	3.4/5.7	MS, L
66	1109	Nonanal	0.0/1.6	0.6/1.9	0.0/2.2	9.6/15.2	15.7/16.6	13.2/8.1	10.2/7.6	9.6/8.2	7.2/5.8	3.7/5.9	6.7/13.1	4.6/4.4	7.0/7.7	0.6/0.6	4.4/5.9	MS, L, KI
67	1122	2-Ethyl hexanoic acid					0.0/0.4	0.8/0.0		0.0/0.7	0.7/1.1	0.5/1.4	0.0/0.4	0.0/0.3	0.0/1.3	0.7/0.7	0.3/0.4	MS, KI
68	1131	4-Methyl-2,7-octadiene												0.2/0.2				MS
69	1149	Benzylnitrile	21.7/21.6	20.8/26.4	7.5/19.0	0.1/0.3	2.1/1.8	2.8/1.0	11.6/8.5	3.2/2.4	1.3/0.9	3.4/2.5	2.2/1.9		2.5/1.3	5.7/5.6	2.1/1.1	MS, L
70	1154	4-Oxoisophorone							0.9/1.2		1.3/1.7		2.1/1.0	0.2/0.2				MS, L
71	1156	Lilac aldehyde C		0.8/1.0		4.1/4.7	1.3/0.9	2.9/2.0	1.9/1.6	1.6/0.9	0.7/1.9	0.0/0.5	1.9/1.0	0.2/0.5	0.0/0.9	1.2/1.0	1.2/0.9	MS, L
72	1169	Benzoic acid						1.4/1.8			1.8/4.4	1.0/0.5	13.6/1.2	5.4/3.3		0.9/0.8	0.7/0.0	MS, L, KI
73	1171	Lilac aldehyde D				1.9/2.4	0.6/0.6		0.0/0.7	1.7/0.8								MS, L
74	1177	Octanoic acid	0.0/1.3	0.8/0.7	0.0/3.1	0.9/1.0	2.3/1.0	2.8/2.4	2.1/1.6	3.9/3.4	2.4/3.6	2.0/6.9	3.4/4.9	1.8/1.3	2.4/5.1	3.6/3.1	0.5/0.9	MS, L, KI
75	1186	trans-Sabinenehydrate												3.1/0.4				MS
76	1192	*p*-Cymen-8-ol											0.0/0.4	1.7/2.5	0.8/2.1	0.8/0.6		MS, L, KI
77	1193	Verbenone												2.5/3.3				MS, L, KI
78	1198	*p*-Menth-1-en-8-ol				0.5/1.1								0.1/0.1				MS, L, KI
79	1200	Dodecane				0.5/0.0				1.3/1.0		0.9/0.0	0.0/2.6					MS, L, KI
80	1204	1,3,8-*p*-Menthatriene												0.7/1.1				MS, L
81	1210	Decanal	0.0/0.8	0.0/0.6		1.0/0.9	2.0/1.5	1.6/1.4	2.1/1.8	2.0/1.7	1.3/1.7	0.6/1.2	1.5/2.9	1.1/1.0	2.1/2.4	2.3/2.1	0.2/0.8	MS, L, KI
82	1212	*p*-*sec*-Butyltoluene												2.3/1.6				MS
83	1215	Isopropyl phenylacetate	1.7/1.5	1.2/2.3	5.3/5.4		1.5/0.8		1.4/1.2							0.3/0.3		MS, L
84	1219	2,3-Dimethylbenzofuran				0.3/0.1												MS, KI
85	1226	*p*-Ethyl-cumene				0.2/0.2								1.0/1.1			0.3/0.7	MS
86	1228	Bornylene															0.2/0.4	MS, L
87	1248	*o*-Anisaldehyde												3.2/2.3				MS, KI
88	1272	Nonanoic acid	0.0/1.2	1.1/1.0	0.0/2.6	1.0/0.9	3.7/1.9	2.1/2.3	1.2/1.6	0.9/0.9	1.8/2.2	1.4/1.0	3.0/2.4	1.2/0.7	1.3/4.4	2.3/2.0	0.5/0.5	MS, L, KI
89	1297	*p*-Cymen-7-ol				0.2/0.4								1.0/0.2				MS, L, KI
90	1304	Carvacrol												0.7/0.8	0.0/0.4			MS, L, KI
91	1310	5,9-Dimethyl-1-decanol														0.7/0.6		MS
92	1368	Decanoic acid				0.2/0.0												MS, L
93	1386	β-Damascenone											0.0/0.3	0.0/0.5		0.0/0.5		MS, L, KI
Changes of the total peak area after 3 months storage,%			−13.3	−16.5	−39.9	−71.2	−61.0	−4.3	−33.3	+29.8	−0.7	−36.8	−15.9	−6.6	+20.2	+14.8	−42.0	

KI – Kovats index, MS – mass spectra, L – literature data, ND – not determined, tr = <0.05%. SD was in the range of 3–10%, with a few exceptions.

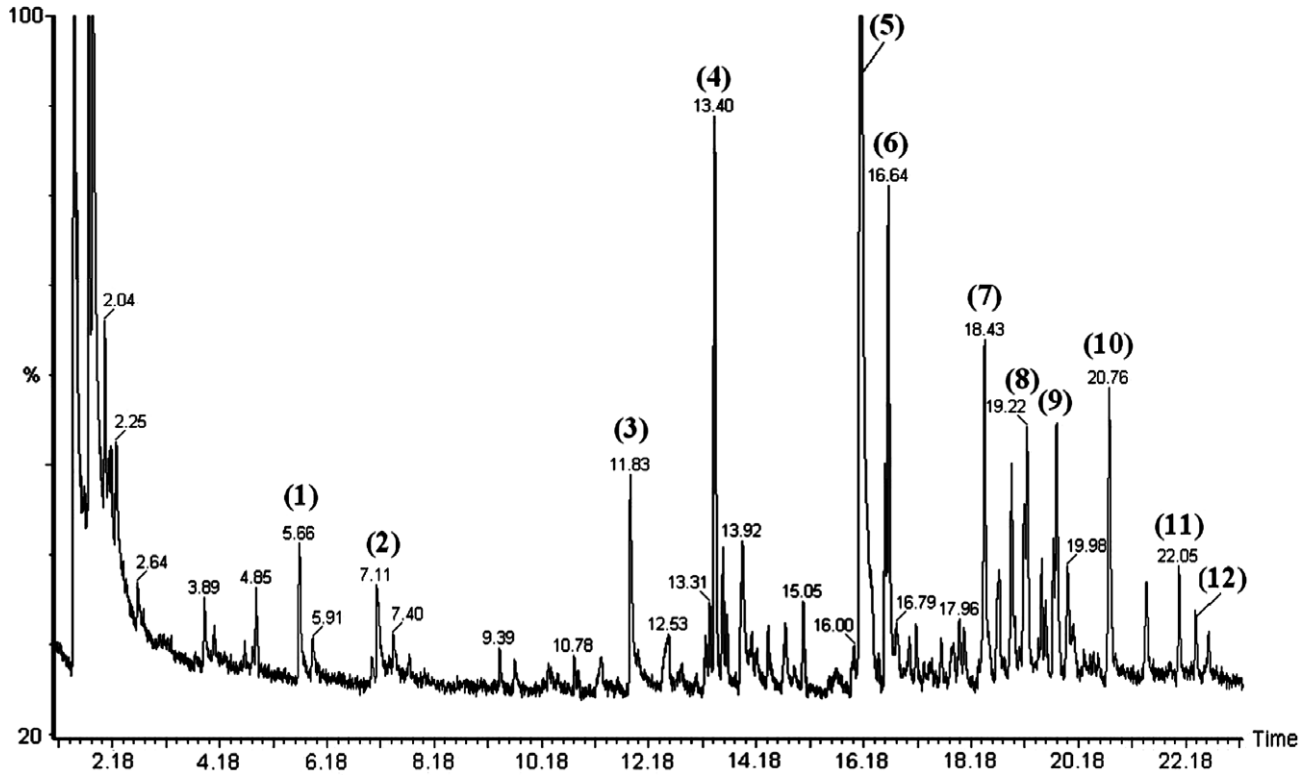

Fig. 1. Typical GC–MS chromatogram of SPME fraction isolated from unifloral rape honey (SR12) by CAR/PDMS fibre (1 – 2,4-dimethylheptane, KI = 803; 2 – furfural, KI = 844; 3 – benzaldehyde, KI = 969; 4 – *cis*-dehydrohy linalool oxide, KI = 1012; 5 – *p*-cymenene, KI = 1093; 6 – nonanal, KI = 1109; 7 – benzoic acid, KI = 1169; 8 – *p*-cymen-8-ol, KI = 1192; 9 – decanal, KI = 1210; 10 – *o*-anisaldehyde, KI = 1248; 11 – *p*-cymen-7-ol, KI = 1297 and 12 – carvacrol, KI = 1304).

blooming. Consequently, some of these compounds may originate from lime tree honeydew.

Radovic et al. (2001) analysed honey from The Netherlands, Portugal, Spain, Denmark, Germany, Italy, France and England and based on their findings suggested that the presence of dimethyl disulphide and the absence of 2-methyl-propanol may be used as a marker of rape honey; however, in our study dimethyl disulphide was detected only in six out of eleven rape honey samples, while 2-methyl-propanol was absent. This finding shows that due to possible peculiarities in the formation of secondary metabolism products in plants growing in different regions the compositional markers for honey may be rather different.

Several short-chain nitrogen containing compounds, namely 2-methylpropanenitrile, 2-methylbutanenitrile, pentanenitrile, 4-methylpentanenitrile, hexanenitrile, benzylnitrile and octanenitrile were found in the analysed samples. Some of them were abundant; pentanenitrile and benzylnitrile were identified in 15 out of 16 samples. Most recently Soria, Martínez-Castro, de Lorenzo, and Sanz (2008) reported high amounts of branched nitriles in *Taraxacum* labelled honeys. Piasenzotto et al. (2003), Soria et al. (2003), De la Fuente et al. (2005), Pontes, Marques, and Câmara (2007) also determined nitrile derivatives in honey samples. For instance, Piasenzotto et al. (2003) identified C5, C6 nitriles in dandelion honey, phenyl acetonitrile in thyme honey. Soria et al. (2003) found small amounts of acetonitrile in the honeydew and heather honeys, while C_4H_5N was present in multiflower honey. Benzylnitrile was identified in a half of the analysed multifloral honey samples from Madeira Island (Pontes et al., 2007). De la Fuente et al. (2005) detected small amounts of acetonitrile, 3-methylbutanenitrile and benzeneacetonitrile in their study of 110 Spanish honeys, while in the most recent study (De la Fuente et al., 2007) they found high proportions of a compound with formula C_4H_5N (18.9% and 27.0%) in the two samples of loquat (*Eriobotrya japonica*) honey. The content of nitrile derivatives in honey samples analysed in our study varied from trace (0.09%) to comparatively high (21.7%) percentages (Table 2); the highest content of benzylnitrile was found in polyfloral (POL1) and unifloral rape (WR2) honeys, 21.7% and 20.8%, respectively.

Benzaldehyde has been reported as an important constituent of honey aroma in numerous studies. It was a major constituent (21.4%) in the volatile fraction of caraway honey (CAR4) analysed in our study, while in other honey samples its content was remarkably lower, from 1.1% (SR13) to 9.5% (WR3). In general, this is in agreement with many previously published data. For instance, Soria et al. (2003) determined from 1.5 to 7.3% of benzaldehyde in their tested honey samples. High content of benzaldehyde was reported in rosemary (13.4%), heather (10.3%) and willow honey 22.3% (De la Fuente et al.,2005, 2007), while in the volatile fraction of citrus origin honey it constituted 1.69–5.63% (Alissandrakis et al., 2007; De la Fuente et al., 2005). It is worth noting that lilac aldehydes usually constitute the largest part of citrus honey volatiles and therefore they were suggested as a marker for such honey (Alissandrakis et al., 2005, 2007; Soria et al., 2003). Lilac aldehyde was detected in ten honey samples from Lithuania and its percentage concentration varied from 0.6% (SR12) to 4.2% (CAR4). For comparison, the isomers of lilac aldehyde in citrus honey were 8.93–13.15% (Soria et al., 2003) and even higher, 10.30–21.86% (Alissandrakis et al., 2007). In other previous studies of honeys, these compounds were in considerably lowers amounts: orange 3.0–5.2% and multifloral 2.4–5.1% (Soria et al., 2003), rosemary 1.49–2.77% and eucalyptus 0.65–1.15% (De la Fuente et al., 2005).

The amount of benzeneacetaldehyde, which is frequently found among honey volatiles (Bentivenga, D'Auria, Fedeli, Mauriello, & Racioppi, 2004; De la Fuente et al., 2005) was higher in POL1 and WR2 honeys, 15.2% and 14.3%, respectively; in other samples its percentage was from 0.4% to 7.1% to (Table 2).

Nonanal was important in unifloral white clover (WCL5) and rape honey (SR6), where it constituted 15.7% and 13.2% respectively; in other honey samples its content varied in a wide range, from 0.6% to 10.2%.

The amount of furfural in the honey samples from Lithuania was from 0.8% (SR14) to 25.5% (POL15). It is known that the content of furfural depends on heat treatment (De la Fuente et al., 2007); therefore, the content of furfural in the isolated fraction of volatile compounds by SPME may be different from that, which is naturally present in honey headspace, because heating was not excluded from the analysis procedure. Furfural was found in various honeys studies by other researchers (Castro-Vázquez, Díaz-Maroto, & Pérez-Coello, 2007; Piasenzotto et al., 2003; Pontes et al., 2007). Ethanol was detected in some honey samples (POL1, WR2, WR3, CAR4, SR6, SR11, SR13, SR14, and POL15), its presence may be related to the development of yeasts in the carbohydrate rich product (De la Fuente et al., 2007).

Several compounds were detected in only one of the tested samples and they might be of a particular interest for the honey authenticity. Such constituents as pentanal, 1,4-pentanediol, 2,3-dihydro-5-methylfuran, 4-pentenal, 2-octene, decane, 2,3-dimethylheptane, limonene, 2,3-dimethylbenzofuran and decanoic acid were found only in caraway honey (CAR4); heptane, 1-pentanol, butanoic and pentanoic acids, 2-methyl-1-pentanol, *p*-cresol, bornylene were detected only in POL15 honey sample. The presence of the remarkable amount of buckwheat pollen (15%) in the latter honey might be one of the reasons for the detected differences in the composition of individual compounds. The contents of the above mentioned compounds were very low (less than 0.7%), except for pentanal, 2,3-dihydro-5-methylfuran, 2-octene, 4-butoxy-1-butene, pentanoic acid, *trans*-sabinenehydrate, verbenone, *p-sec*-butyltoluene, *o*-anisaldehyde (>1.6%). Therefore, minor compounds may be present in the other analysed honey samples at the concentrations below GC detection threshold.

The composition volatile fraction of SR12 honey (92.6% of rape pollen) also was rather different as compared to other analysed honey samples. Hexanal, 4-methyloctane, cumene, 3-caren-2-ol, β-phellandrene, 4-methyl-2,7-octadiene, 2,6-dimethyl-3,5,7-octatriene, *trans*-sabinene hydrate, verbenone, 1,3,8-*p*-menthatriene, p-sec-butyltoluene, *o*-anisaldehyde, carvacrol were identified only in this honey. This honey is also distinguished by a high percentage (37.9%) of *p*-cymenene, which was also abundant in SR13 (12.3%); in other honey samples the content of this compound was remarkably lower, 0.5–2.8%.

The following compounds were found in less than 3 honey samples: chloroform (SR7, SR8), 3-methylhexane (SR11, POL15), 2-methyl propanoic acid (WR1, CAR4, WCL5), 2,3-dihydroxypropanal (CAR4, SR14), hexanenitrile (WR3, CAR4, SR6), 4-butoxy-1-butene (WR2), 2-methyl butanoic acid (POL1, SR7), *p*-cymene (SR12, SR13, SR14), *trans*-linalool oxide (CAR4, SR8, POL15), p-ethyl-cumene (CAR4, SR12, POL15), *p*-cymen-7-ol (CAR4, SR12), 5,9-dimethyl-1-decanol (SR14) (Table 2). As it was mentioned above, some compounds may be artefacts due to heating and yeast contamination. It is possible that the origin of chloroform which was found in the two samples at the concentration of 0.2% and 2.8% was the environmental or other pollution.

3.2. Volatile compounds of beebread

The composition of volatile compounds of beebread was slightly different from the composition of volatiles in honey. The difference may be influenced by the unique composition of the beebread. Beebread is a mixture of honey and pollen, therefore it is likely that pollen volatile compounds may play more important role for beebread than for honey. However, there is a lack of data on the beebread volatiles in the literature, and it is not possible to compare our results with any previously obtained. Dimethyl sulphide, pentanenitrile, furfural, benzaldehyde, nonanal, benzylnitrile, and decanal were identified both in beebread and honey, while 2-methylbutanenitrile, 3-methyl pentanoic acid, benzenacetaldehyde, linalool, octanoic acid, which were present in the majority of honey samples were not detected in the headspace of beebread (Table 3). The chromatographic profile of beebread volatiles is presented in Fig. 2, while the composition of volatiles is listed in Table 3. It can be clearly observed that the largest peaks were attributed to dimethyl sulphide, acetic acid, furfural, nonane and 1-heptadecene; the percentage of these compounds was 20.0%, 13.4%, 9.8%, 10.4% and 13.9%, respectively. The amount of benzaldehyde in beebread was very low, 0.9%, while in 10 out of 15 honey samples the content of this compound was > 5.0%. Beebread differs from honey samples also in the content of acetic acid, 1-phenylpropan-2-ol, 3-furaldehyde, 2-heptanone, 4-ethyl-4-methyl-1-hexene, 5-hydroxymethyl furfural, tridecane, 1-heptadecene.

3.3. Changes of volatile compounds in honey during storage

The effects of storage on the composition of volatile compounds were studied for many foods, however the information on possible changes of aroma compounds in honey are very scarce, while physical changes of honey during storage are evident and can be followed visually. In general, the changes of food volatile constituents occur due to the transformation of some compounds to others due to oxidation, fermentation (microbiological purity is important), thermal processing, storage conditions and some other factors. In our study, the same honey samples were tested after three months of storage at room temperature. SPME–GC/MS results showed that the composition of volatile compounds after storage was slightly

Table 3
Volatile compounds of beebread

No.	KI	Identified compound	Relative percentage,%	Source of identification
1	ND	Ethanol	0.6±0.1	MS, L
2	ND	Dimethyl sulphide	20.0±1.8	MS, L
3	ND	Acetic acid	13.4±0.9	MS, L
4	ND	Pentanenitrile	0.6±0.1	MS, L
5	ND	Dimethyl disulphide	3.0±0.2	MS, L
6	ND	1-Phenylpropan-2-ol	0.4±0.0	MS, L
7	803	2,4-Dimethylheptane	3.9±0.1	MS, L
8	809	2-Octene	0.5±0.0	MS, L, KI
9	844	Furfural	9.8±0.3	MS, L, KI
10	849	3-Furaldehyde	0.7±0.1	MS, L
11	853	2-Hepten-1-ol	2.0±0.1	MS, L
12	897	2-Heptanone	0.4±0.0*	MS, L, KI
13	900	Nonane	10.4±0.7	MS, L, KI
14	970	Benzaldehyde	0.9±0.1	MS, L, KI
15	974	Dimethyl trisulphide	0.5±0.1	MS, L
16	990	Hexanoic acid	3.2±0.3	MS, L, KI
17	1000	Decane	0.8±0.1	MS, L, KI
18	1008	Octanal	0.9±0.1	MS, L, KI
19	1043	Benzyl alcohol	0.5±0.0	MS, L, KI
20	1079	Heptanoic acid	0.4±0.0	MS, L, KI
21	1093	*p*-Cymenene	0.3±0.0	MS, L, KI
22	1100	Undecane	1.1±0.1	MS, L, KI
23	1105	Hotrienol	0.2±0.0	MS, L, KI
24	1108	Nonanal	1.2±0.1	MS, L, KI
25	1148	Benzylnitrile	1.0±0.1	MS, L
26	1169	Benzoic acid	1.3±0.1	MS, L, KI
27	1200	Dodecane	4.4±0.3	MS, L, KI
28	1210	Decanal	0.7±0.1	MS, L, KI
29	1229	4-Ethyl-4-methyl-1-hexene	0.5±0.0	MS
30	1236	5-Hydroxymethylfurfural	2.5±0.2	MS
31	1299	Tridecane	0.7±0.1	MS, L, KI
32	1492	1-Heptadecene	13.9±1.1	MS, L, KI

KI – Kovats index, MS – mass spectra, L – literature data, ND – not determined, * SD < 0.05.

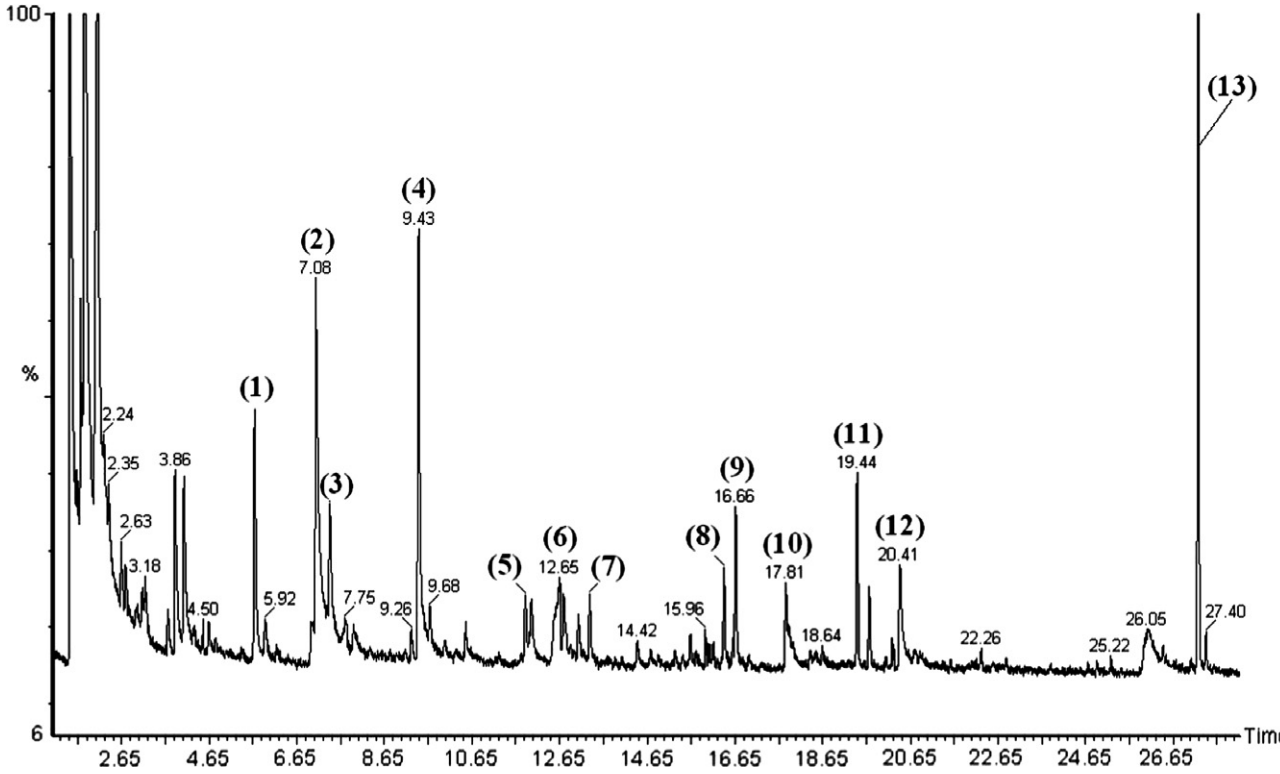

Fig. 2. Chromatographic profile of beebread analysed by SPME–GC–MS (1 – 2,4-dimethylheptane, KI = 803; 2 – furfural, KI = 844; 3 – 2-hepten-1-ol, KI = 853; 4 – nonane, KI = 900; 5 – benzaldehyde, KI = 970; 6 – hexanoic acid, KI = 990; 7 – octanal, KI = 1008; 8 – undecane, KI = 1100; 9 – nonanal, KI = 1108; 10 – benzylnitrile, KI = 1148; 11 – dodecane, KI = 1200; 12 – 5-hydroxymethylfurfural, KI = 1236 and 13 – 1-heptadecene, KI = 1492).

different as compared to a more fresh honey. For instance, some compounds present in fresh honey were not detected after three months of storage, while some new compounds were found. For instance, in a few honey samples dimethyl sulphide, 2-methylbutanenitrile, dimethyl disulphide, hexanal, nonane, dimethyl trisulphide, octanal, heptanoic acid, *p*-cymenene, hotrienol, nonanal, lilac aldehydes C and D, *p*-cymen-8-ol, decanal, nonanoic acid, carvacrol, β-damascenone were detected only after three months of storage. It was suggested that several aldehydes and ketones formed by the oxidation of fatty acids, particularly linoleic and linolenic, may be of importance for the development of rancid flavour (Overton & Manura, 1999). On the contrary, for some other honeys, isobutane, hexane, 3-methylbutanal, 2-methyl propanoic acid, (2-methylpropyl)-benzene, 2,4-dimethylheptane, 2-methyl butanoic acid, 2-nonanone, undecane and decanoic acid were detected only in fresh samples (Table 2).

Percentage composition of honey volatile compounds also has changed during storage. It was reported that octane concentration increases with time during storage (Overton & Manura, 1999). In our study the content of octane increased during storage in 4 out of 5 honey samples, while the percentage in the 5th sample did not change. Figs. 3–5 show the changes of a percentage composition of benzaldehyde, benzeneacetaldehyde and linalool in the analysed honey samples. It can be observed that after three months the content of benzaldehyde and benzenacetaldehyde decreased in 8 out of 15 samples, while the content of linalool increased in 8 out

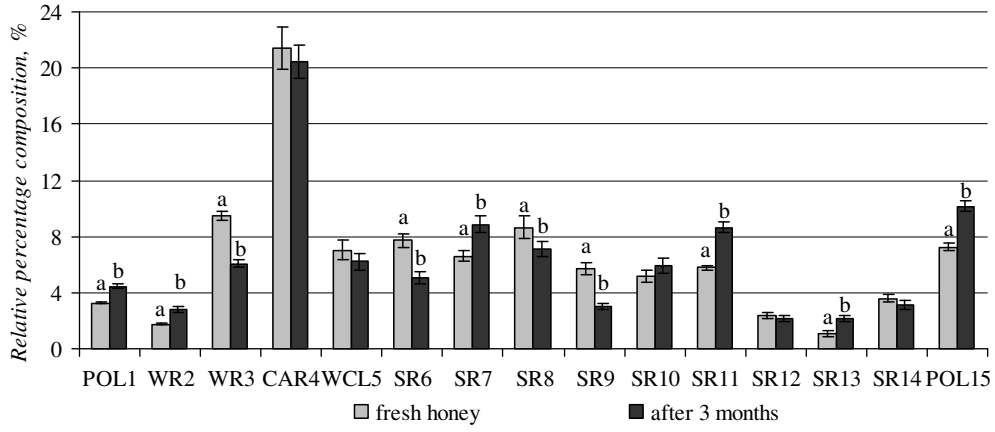

Fig. 3. Changes of percentage composition of benzaldehyde in the volatile fraction of honey samples during storage: "a" and "b" indicate significant difference ($p < 0.05$) in the amount of benzaldehyde.

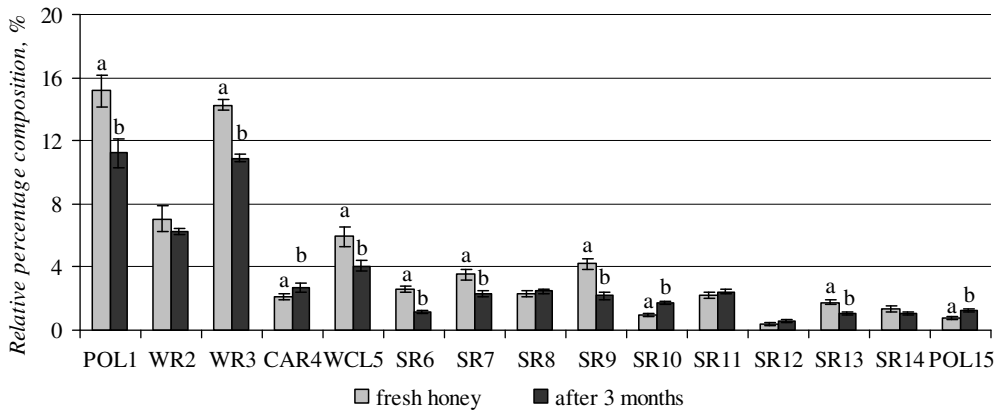

Fig. 4. Changes of percentage composition of benzenacetaldehyde in the volatile fraction of honey samples during storage: "a" and "b" indicate significant difference ($p < 0.05$) in the amount of benzenacetaldehyde.

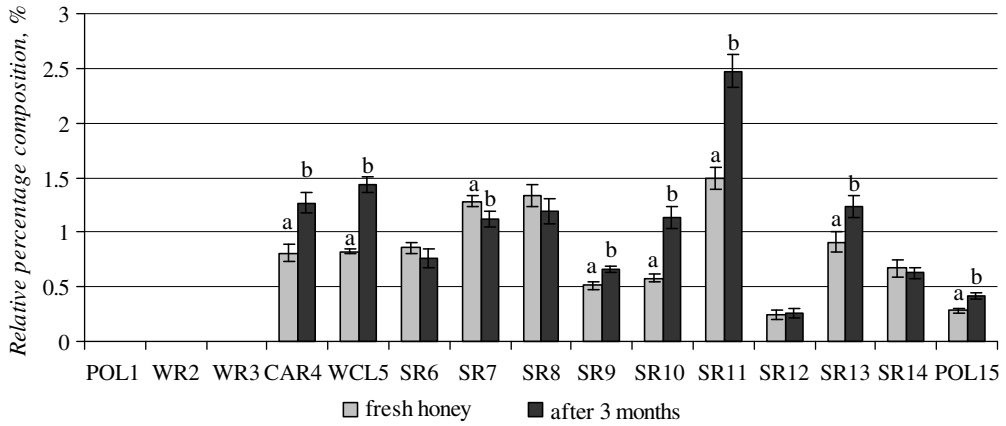

Fig. 5. Changes of percentage composition of linalool in the volatile fraction of honey samples during storage: "a" and "b" indicate significant difference ($p < 0.05$) in the amount of linalool.

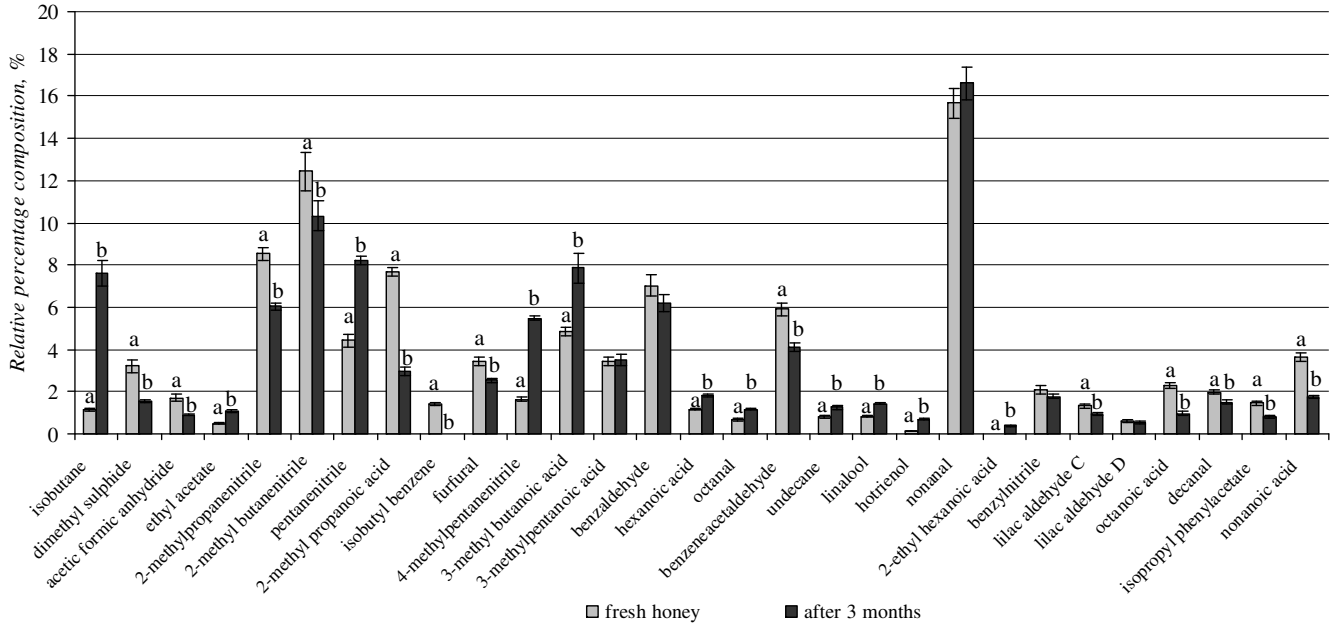

Fig. 6. Changes of percentage composition of identified compounds in WCL5 honey during storage; "a" and "b" indicate significant difference ($p < 0.05$) in the amount of corresponding compounds.

of 12 samples (Fig. 5). Fig. 6 shows the changes of identified compounds in unifloral white clover honey (WCL5). 2-methyl propanoic acid, isobutylbenzene, octanoic acid and decanal were not detected after three months in this honey, while some new constituents, namely ethyl acetate, hotrienol, 2-ethyl hexanoic acid, lilac aldehydes C and D were identified in the stored honey. The changes of identified compounds in the analysed honey samples during storage are listed in Table 2.

In order to preliminary assess the scope of the changes of honey volatiles, the ratio of the total integrated peak areas was calculated. In many cases, as judged from this relative characteristic, the loss of volatiles was determined. The highest loss of volatiles accounting for approximately 70% was found in CAR4 and WCL5 honeys, while the amount of volatiles in SR9 honey remained unchanged. In 9 honeys the changes of the total peak areas during storage was from 4.3% to 42.0%. On the other hand, the total amount of volatiles from SR8, SR13 and SR14 honeys increased during storage by 29.8%, 20.2% and 14.7%, respectively. It should be noted that the results on the changes of honey volatile compounds during storage can be considered as preliminary ones. So far as headspace volatiles were measured, the changes in physical state of honey, e.g consistency, rheological properties, and crystallisation may play a crucial role in the release of volatile compounds. However, the relationships between physical properties and flavour release were beyond the scope of this study.

4. Conclusions

Remarkable variations in the volatile profiles of Lithuanian honey samples of monofloral (rape, caraway and white clover) and polyfloral origin were established by SPME–GC/MS. Although all honey samples contain the same chemical classes of identified compounds (alcohols, aldehydes, acids, linear and branched hydrocarbons, terpenes, ketones, nitrile and furfural derivatives) their quantitative and qualitative composition were different. However, in most cases it was difficult to assign precise volatile compounds, which could serve as indicators of floral sources of honey. Unifloral caraway honey was characterised by a high amount of benzaldehyde (21.4%), which was 2.3–19.5 times higher than in the other analysed honeys. One sample of spring rape honey contained high percentage of *p*-cymenene (37.9%); its content in the majority of other samples varied from 0.4% to 2.9%. Beebread volatile profile differed from honey profiles, particularly by a high percentage of acetic acid and 1-heptadecene.

Remarkable changes in the content and the composition of volatile compounds occurred for the majority of honey samples during 3 months storage. Total amount of headspace volatiles of caraway and white clover honeys decreased by approximately 70% after three months; in some other honeys the decrease was less considerable. On the other hand, in the three unifloral spring rape honey samples after their storage the content of SPME volatiles was found to have increased by 14.7–29.8%. Most likely, two main reasons may be responsible for the changes of honey headspace volatile composition during storage: direct chemical changes of honey composition (e.g., formation of new compounds, loss of some volatile components) and physical changes which may have remarkable influence on the release of aroma constituents.

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