Key odour-active compounds in selected Slovakian poppy seed (*Papaver somniferum* L.) varieties revealed by gas chromatography-olfactometry

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Summary

Seeds obtained from three Slovakian poppy varieties (*Papaver somniferum* L.): white seed variety Albín, and blue seed varieties Gerlach and Malsar, were investigated with the aim to detect and identify odour-active compounds in their volatile fractions, and to evaluate contributions of individual odorants to the overall odour of seeds. For the isolation of volatile fraction, headspace solid-phase microextraction (HS-SPME) was used. Analysis of volatiles was carried out by gas chromatography-mass spectrometry (GC-MS) and, in parallel, by gas chromatography coupled to the flame ionization detection and olfactometry (GC-FID/O). A total of 23 odour-active compounds were detected in the HS-SPME extracts, and 17 of them were identified by a combination of several independent methods. According to their relative contents, hexanal, hexanol, 2-pentylfuran, hexanoic acid and pentanol represented most abundant volatiles. Nevertheless, on the basis of odour descriptions and high odour intensities, limonene, nonanal, unknown compound with strong balsamic, valeriana oil-like odour, 2-pentylfuran, pentanol and hexanal were found to be principal odour-active compounds in the volatile fraction of all varieties. Odour profile of white seed variety was superior to those obtained for blue seed varieties in the total number, relative content and odour intensity of volatiles, and proved the richest and well-balanced odour, partially evoking walnut aroma.

Keywords

poppy seed; volatiles; aroma analysis; solid phase microextraction; gas chromatography-olfactometry

Ordinarily, the poppy plants (*Papaver somniferum* L.) are divided into two groups: the opium poppies producing high amounts of latex and compounds of alkaloid type, and poppies with a low content of alkaloids, which are useful for food applications. In Slovakia, breeding of poppy began after the Second World War. The only institute active in this field is Research and Breeding Station at Malý Šariš, which currently belongs to Research Institute of Plant Production, Piešťany. Firstly, aim of the production of new poppy plant materials was detection and evaluation of the regional varieties, followed by inter-varietals crossing, induced mutagenesis, distant hybridization and polyploidization. However, only by crossing were achieved desired results, i.e. materials with high yield of seeds and blue colour of seed with a relatively high content of morphine for the pharmaceutical purposes. Nevertheless, the next social order was a variety for food purposes, with a minimum of morphine in the seed and white colour of seed as a suitable alternative of nuts. Nowadays,

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besides the white seed variety (namely Albín), the grey and blue varieties in various shades, and also ochre colour varieties of seed are known [1–3].

Despite the long tradition of poppy cultivation in Slovak Republic resulting in the development of several valuable varieties [2, 3], poppy seed production has at present mostly local character. In 2014, total harvested areas represented only 1 704 ha, which is approximately 1 % of the world production [4]. However, varieties of Slovakian origin are successfully registered and grown in the neighbouring Czech Republic, which currently belongs to the most important producers of poppy seed worldwide [4]. Because of their low to medium morphine content in dry capsules (0.3–0.6 %) [2], they are labelled as food cultivars and are suitable for direct consumption.

Poppy plant is an annual industrial crop, which is grown either for the production of opium or oilseeds, within the meaning of the previous text [5–7]. Poppy seeds are used in traditional cuisine of several nations, mostly in confectionary and bakery food products such as fillings in cakes and desserts, or sprinkled on bread or rolls [5, 6]. Moreover, they are a source of highly valuable oil, which is used not only for culinary purposes but also as an adjuvant for pharmaceutical and medical diagnostics, or as a component of cosmetic products and high-class oil-paints or varnishes [7].

In a Turkish study [5] focused on chemical composition of seeds obtained from seven poppy varieties, content of crude oil, protein and fibre were established between 32.4 % and 45.5 %, 11.9–13.6 % and 4.9–6.3 %, respectively. Besides that, high contents of some biologically important minerals, such as Ca, K, P, Mg, Na or Fe, and appreciable amount of tocopherols (378.20–597.30 $mg \cdot kg^{-1}$) were reported. In a similarly oriented study [6], considerably high amount of phytosterols (1099.40–4810.16 $mg \cdot kg^{-1}$) was additionally determined, β-sitosterol, campesterol and Δ^5-avenasterol being found to be major components of the sterol fraction. Poppy seed quality was investigated also by other research groups [1, 8–10] and, in general, similar results as those mentioned above were obtained. Although most of the studies were primarily focused on fatty acid and tocopherol determination, they all proved that the analysed poppy seeds, and particularly their oils, represent foodstuffs with a high nutritional value, suitable for processing of health-promoting food products.

Besides the above mentioned positive properties and consequent health benefits of poppy seed consumption, it is also a unique aroma that contributes to the great popularity of poppy products among consumers. Several studies [7, 11–14] were focused on the composition of poppy seed oil volatile fraction and on effects of different production technologies on its organoleptic quality.

In 1990, Li et al. [11] reported on detailed chemical composition of oil extracted from ground poppy seeds for the first time. The authors found that poppy oil contains hydrocarbons, aldehydes, ketones, oxygenated derivatives of aromatic and furan compounds as well as some organic acids and ester compounds.

Later, Krist et al. [7] studied volatile constituents of oils obtained by pressing white, blue and grey poppy seeds at different conditions. They detected a total of 30 volatiles in the oils, most of them representing oxidative products of lipids, and found 1-pentanol, 1-hexanal, 1-hexanol, 2-pentylfuran and caproic acid to be characteristic components. Both qualitative and quantitative composition of volatiles was considerably influenced by pressing methods, while it remained unaffected by storage time.

The same authors published another study [12] focused on the detection of poppy seed oil adulteration with sunflower oil at different levels (5–40 %). On the basis of analysis of volatiles, α-pinene was chosen as a suitable indicator of a sunflower oil admixture in all relevant amounts.

In the study of Guo et al. [13], the flat and full aroma of poppy seed oils were compared in order to investigate causes of their sensorial differences at the molecular level. Authors concluded that pyrazine compounds could play an important role in the formation of flavour of full aroma oil, while 2-decenal and mainly 2,4-decadienal seem to be major components responsible for sour and rancid flavour of the flat aroma oil.

Impact of different pre-treatments (roasting or enzyme treatment) on the organoleptic quality of poppy seed oils obtained by cold pressing technique was studied by Emir et al. [14]. The flavour profile analysis (FPA) proved that the pretreatments applied prior to the cold processing were effective in changing the sensory properties of the poppy seed oils. Seed roasting was identified as the best processing operation in terms of improveing the quality of poppy seed oils. In addition, 75 volatiles were quantified in this study but no clear relationship was found among 12 sensory descriptive terms developed for FPA and the concentrations of volatiles present in the oils.

All above mentioned studies were carried out using predominantly the technique of solid phase microextraction (SPME) [7, 12, 14] or the method of simultaneous distillation-extraction (SDE) [13] for isolation of volatiles, which were followed by

gas chromatography-mass spectrometry (GC-MS) analysis. Analysis using GC-MS allows the resolution and identification of a majority of volatile compounds that are present in an odour. However, a large GC peak area, generated by instrumental detector, does not necessarily correspond to high odour intensity because of the large differences in odour thresholds of individual volatiles causing that one compound can be sensorially perceived at much lower or higher concentrations than another one. Consequently, concentrations of volatiles obtained by GC-MS do not necessarily reflect real contributions of volatile compounds to the overall aroma of food [15, 16]. In such a case, a combination of instrumental and sensory analysis in the form of gas chromatography-olfactometry (GC-O) provides the selectivity to detect only those compounds in a sample, concentration of which is above their odour threshold, and determine their intensity and odour quality at a given concentration [15].

From this perspective, there is still limited information on sensorial relevance of individual poppy seed volatiles. Hence, the aim of this study was to characterize quality of seeds originating from several Slovakian poppy varieties in terms of their volatile composition and investigate contribution of individual volatiles to the overall organoleptic properties of poppy seeds by GC-MS and GC-FID/O methods.

MATERIALS AND METHODS

Samples

Seeds from seven Slovakian poppy varieties were provided by Plant Production Research Centre, Piešťany – Research and Breeding Station at Malý Šariš (Slovakia). These were blue seed varieties Maraton, Opal, Gerlach, Malsar, Major, Bergam, and one white seed variety Albín. On the basis of preliminary GC-MS study of all seven varieties (data not shown), only Albín, Gerlach and Malsar were chosen for further investigation by GC-FID/O method. Prior to the extraction of volatiles, poppy seed was always ground in kitchen grinder to achieve better release of volatile fraction from matrix.

Chemicals

All chemicals used as reference standards for identification purposes of volatiles (listed in Tab. 1) were gifts donated from Bedoukian Research (Danbury, Connecticut, USA) and French National Institute for Agricultural Research (INRA) Laboratories (Dijon, France).

Headspace solid-phase microextraction (HS-SPME)

Each sample (5 g) was incubated statically in a 40 ml vial in a metallic block thermostat (Liebisch, Bielefeld, Germany) at 50 °C for 30 min, with an SPME fibre placed in the headspace. The SPME fibre covered with polymeric coating of divinylbenzene (DVB), carboxen (CAR) and polydimethylsiloxane (PDMS) - DVB/CAR/PDMS, 2 cm, "For odours", film thickness 50/30 μm, (Supelco, Bellefonte, Pennsylvania, USA) was used. The fibre was initially conditioned by heating in the GC injector at 270 °C for 1 h. SPME extracts were desorbed at 250 °C in the GC injector during the entire analysis. This setup was chosen on the basis of preliminary optimization of SPME procedure, performed in order to achieve the best recovery of volatile fraction. Four types of fibre stationary phase (65 μm PDMS/DVB, 75 μm CAR/PDMS, 70 μm CAR/DVB, 50/30 μm DVB/CAR/PDMS) as well as three different extraction temperatures (30 °C, 40 °C and 50 °C) and four durations (15, 30, 45 and 60 min) were tested within this step.

Gas chromatography-mass spectrometry (GC-MS)

Volatile fractions obtained by HS-SPME were analysed by GC-MS using the gas chromatograph Agilent 6890N (Agilent Technologies, Palo Alto, California, USA) coupled to the mass spectrometric detector 5973 inert (Agilent Technologies) equipped with fused silica capillary column Ultra 1 (50 m × 0.32 mm × 0.52 μm; Agilent Technologies) operating with a temperature programme 40 °C (1 min), 5 °C·min^{-1}, 250 °C (1 min). The linear velocity of the helium carrier gas was 35 cm·s^{-1} (measured at 143 °C). Pulsed splitless injection was used at an injector temperature of 250 °C. Ionization voltage (EI) was set to 70 eV.

Gas chromatography-olfactometry (GC-FID/O)

In parallel with GC-MS, samples were analysed by GC-FID/O using the detection frequency concept of posterior evaluation of odour intensity according to the modified procedure of Janáčová et al. [17].

A sniffing procedure panel was formed of 5 judges (3 women and 2 men; aged in the range of 26–61 years) who were chosen from 11 assessors trained in sensory evaluation. All judges were asked to measure the overall intensity of each odour using a 0–3 scale with seven possible scores (half values allowed). Panel intensities were calculated as arithmetic mean of the measurements of odour intensities obtained from 5 independent measurements. Each sensory perception was based on at least 4 citations. The value of ± 0.5

Tab. 1. List of key odour-active compounds identified in the volatile fraction of poppy seeds.

No	Linear retention index	Compound	Odour description	References
1	667.9	pentanal	pungent, pleasant, yeasty	LRI, MS, ST, OD, LIT
2[c]	–	2-methyl-2-pentenal[a] + unknown[b]	slightly fruity, grassy, green	MS, OD
3	717.0	3-methyl-1-butanol	buttery, tallowy, waxy	LRI, MS, ST, OD, LIT
4	746.9	pentanol	pungent, unpleasant, spoil yeasty	LRI, MS, ST, OD, LIT
5	772.4	hexanal	nutty, fatty, slightly bitter	LRI, MS, ST, OD, LIT
6	822.8	(*E*)-2-hexenal	green, leafy, apple-like, bitter	LRI, MS, ST, OD
7	850.8	hexanol	green apple-like	LRI, MS, ST, OD, LIT
8	962.9	1-octene-3-ol	mushroom-like	LRI, MS, ST, OD, LIT
9	–	unknown[b]	slightly bitter, tart	–
10	967.2	hexanoic acid	sweaty, rancid	LRI, MS, ST, OD, LIT
11	977.5	2-pentylfuran	green bean, metallic, bitter, mousy smell, poppy-like	LRI, MS, ST, OD, LIT
12	–	unknown[b]	gas-like, paper-like	–
13	1012.5	3-octene-2-one	bitter, tart, blueberry note	LRI, MS, ST, OD, LIT
14	1018.1	limonene (unknown enantiomer)	bitter, fruity, citrus peel-like	LRI, MS, ST, OD, LIT
15	1030.0	(*E*)-2-octenal	green-leafy, fatty, nutty	LRI, MS, ST, OD, LIT
16	–	unknown[b]	strong balsamic, valeriana oil-like	–
17	1081.4	nonanal	fatty, bitter, waxy, ground poppy seeds-like	LRI, MS, ST, OD, LIT
18	1132.7	(*E*)-2-nonenal	penetrating fatty, waxy, tallowy, cucumber-like	LRI, MS, ST, OD, LIT
19	–	unknown[b]	fatty, slightly coconut-like	–
20[c]	1182.9 + 1183.4	(*E,E*)-2,4-nonadienal + decanal	fatty, slightly bitter, poppy seeds-like	LRI, MS, ST, OD, LIT
21	1270.7	pentyl hexanoate	esteric, pineapple-like	LRI, MS, ST, OD

a – tentative identification, b – betected only by olfactometry, c – partial overlap of sensory responses.
Compounds were identified on the basis of the following criteria: LRI – linear retention index, MS – mass spectrum, ST – comparison with standard compound, OD – odour quality, LIT – literature reference.

was considered as measurement deviation.

The gas chromatograph Agilent 7890A (Agilent Technologies) coupled to flame ionization detector (FID) and an olfactory detector port ODP3 (Gerstel, Mülheim an der Ruhr, Germany) was used. The capillary column Ultra 1 (50 m × 0.32 mm × 0.52 μm, Agilent Technologies) operated with the temperature programme 40 °C (1 min), 5 °C·min^{-1}, 250 °C (1 min). Hydrogen was used as a carrier gas. Sniffing analyses were carried out at constant pressure, and at linear velocity of 45 cm·s^{-1} (measured at 143 °C). Pulse splitless injection was used at an injector temperature of 250 °C.

The olfactory detection port operated at a temperature of 180 °C, interface temperature was 230 °C, and the flow of added nitrogen in olfactory detector port humidifier was 12 ml·min^{-1}. The sniffing time of each judge was 45 min, and the judges were sniffing during whole run time without interruption.

Identification and semi-quantitative analysis of volatile compounds

The volatiles were identified on the basis of comparison of their linear retention indices (*LRI*), mass spectra (MS), GC analysis of standards and by comparison of data on occurrence and odour description with literature. The linear retention indices were calculated using the equation of van Den Dool and Kratz [18] and C_6-C_{13} *n*-alkanes were used as reference standards. *LRI* data were compared and confirmed with *LRI* data obtained by measurement of standard compounds. For this purpose, our in-house database of *LRI* data was used. Identification of compounds by comparison of mass spectra was performed with Wiley and NIST MS libraries (National Institute of Standards and Technology, Gaithersburg, Maryland, USA). Relative proportions of individual volatile compounds as semi-quantitative parameters were calculated by the method of internal normalization and expressed as percentage.

Given values are the arithmetic means of triplicates.

Statistical analysis

Multivariate statistical analysis of GC-MS experimental data was performed by the software package Unistat 6.0 (Unistat, London, United Kingdom) involving the method of principal component analysis (PCA) in order to define, interpret and visualize the differences between the poppy seed varieties. For this purpose, relative contents of individual volatiles in respective poppy seed samples under study were used.

RESULTS AND DISCUSSION

Optimization of SPME

Preliminary optimization of SPME conditions is in detail described in the experimental part of this study, including type of fibre, extraction temperature and time. The best recoveries of volatiles were achieved with DVB/CAR/PDMS fibre, which was successfully used also by other researches for the extraction of volatiles from poppy seed oil [7, 14]. As regards temperature optimization, value of 50 °C was chosen, because it supported effective release of the volatile fraction from the fat-containing matrix and, at the same time, it did

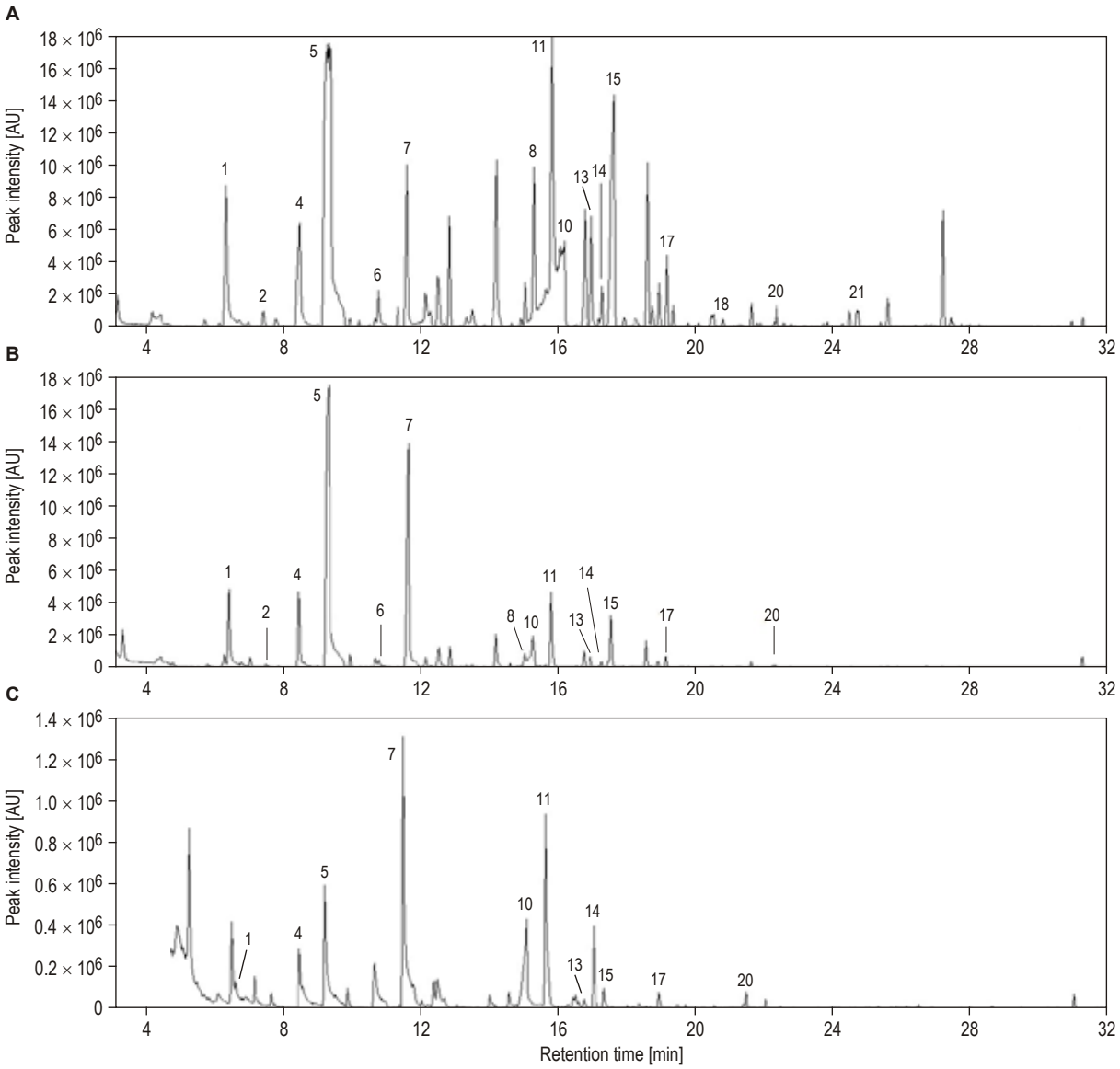

Fig. 1. Chromatograms of volatile fractions of poppy seed varieties obtained by HS-SPME coupled to GC-MS.

A – white seed variety Albín, B – blue seed variety Malsar, C – blue seed variety Gerlach.
Numbering of peaks corresponds to indications of volatiles in Tab. 1.

not induce changes in the chemical composition of samples. With the selected fibre and temperature, the extraction time of 30 min was sufficient to achieve the equilibrium. Moreover, no significant differences in recoveries of volatiles were noticed at the extraction times of 30, 45 or 60 min and thus, the shortest extraction time was chosen from practical reasons (duration of analysis).

Preliminary GC-MS analysis

As it was indicated in the previous parts of this study, seven Slovakian poppy seeds varieties (6 blue and 1 white seed) were analysed by HS-SPME coupled to GC-MS in order to obtain basic information about the composition of their volatile fractions, and potential differences in the number or relative content of individual volatiles (data not shown). These experiments did not show any significant variability within the blue seed varieties. Therefore, only two sorts (Gerlach and Malsar) were chosen as the representatives for further investigation of odour-active components, performed by GC-FID/O. On the contrary, the only one white seed variety Albín proved to be significantly different from all analysed blue poppy seed varieties. As demonstrated in Fig. 1, a number of separated components in the volatile fraction of Albín was approximately by one third higher than that in blue seed varieties and, similarly, the contents of some volatiles were considerably higher than in the case of blue seed varieties.

The statistical processing of experimental GC-MS data by the method of PCA also confirmed uniqueness of Albín compared with blue seed varieties. It should be mentioned here that PCA has recently been successfully used also for differentiation of organic and conventional wines, compositional differentiation of cheeses and evaluation of technological modifications in the fruit juices production [19–21]. Plot of principal components (Fig. 2), constructed on the basis of relative contents of individual volatiles, clearly demonstrated the differentiation of eigenvectors belonging to the blue seed samples and white seed variety, as the respective eigenvectors of white/blue seed varieties were localized in the opposite sections of PCA plot, forming independent clusters. Thus, it is obvious that poppy seed varieties differed significantly in the relative composition of volatile components. Taking into account all the above mentioned Albín characteristics, the variety was also chosen for the GC-FID/O analysis as described below.

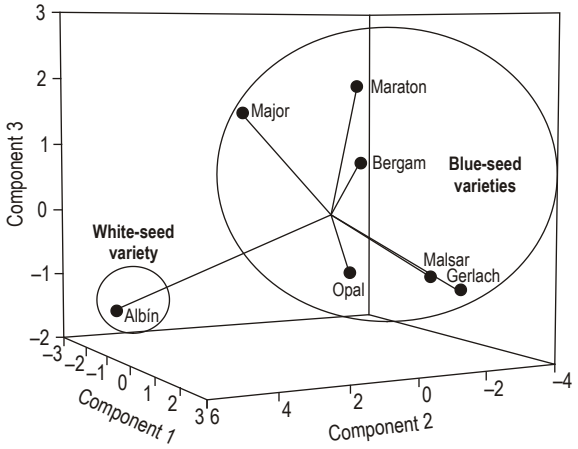

Fig. 2. Plot of principal components.

Differentiation of blue poppy seed varieties from the white seed variety constructed on the basis of the relative contents of individual volatile compounds obtained by the GC-MS.

GC-FID/O study

In general, 23 odour-active compounds were detected in the poppy seeds of three selected varieties by GC-FID/O (Tab. 1). However, only 21 olfactory responses were recorded, due to the overlaps between odours in two cases (2-methyl-2-pentenal + unknown compound, (*E,E*)-2,4-nonadienal + decanal). Seventeen compounds were identified by a combination of independent methods as indicated in experimental part. In one case (2-methyl-2-pentenal), only partial information was available and thus only tentative identification was possible. Five compounds remained unidentified at this stage, because they were detected only by olfactometry. In the future research, the unknown odour-active compounds should be further investigated by determination of their *LRI* on a GC column with stationary phase of different polarity.

The overall aroma of analysed samples of poppy seed was found to be formed by the following volatile odour-active components: nine aldehydes (pentanal, 2-methyl-2-pentenal, hexanal, (*E*)-2-hexenal, (*E*)-2-octenal, nonanal, (*E*)-2-nonenal, (*E,E*)-2,4-nonadienal, decanal), four alcohols (3-methyl-1-butanol, pentanol, hexanol, 1-octene-3-ol), one ketone (3-octene-2-one), organic acid (hexanoic acid), furan derivative (2-pentylfuran), ester (pentyl hexanoate), terpene (limonene) and five unknown compounds. Almost all identified compounds were previously described to be part of poppy seed oil by several research groups [7, 11–14]. Majority of them are known as products of autoxidation reactions of unsaturated fatty acids. According to the research of Hlinková et al. [22] focused on the fatty acid compositions of the same poppy varieties, linoleic

Tab. 2. Relative contents and odour intensities of key odour-active compounds identified in the volatile fractions of poppy seeds.

No	Compound	Poppy variety					
		Albín		Malsar		Gerlach	
		Relative content [%]	Odour intensity	Relative content [%]	Odour intensity	Relative content [%]	Odour intensity
1	pentanal	4.2	2	5.8	2	0.3	0.5
2	2-methyl-2-pentenal + unknown	0.4	1.5	0.2	1	nd	0.5
3	3-methyl-1-butanol	nd	1.5	nd	–	nd	–
4	pentanol	4.0	2	4.7	2	3.5	2
5	hexanal	25.1	2	40.6	2	10.8	1
6	(*E*)-2-hexenal	0.9	1	0.8	0.5	nd	–
7	hexanol	11.6	1	18.4	1	27.5	1
8	1-octene-3-ol	4.0	1.5	0.9	–	nd	–
9	unknown	nd	1.5	nd	–	nd	–
10	hexanoic acid	8.6	2	3.5	0.5	8.6	2
11	2-pentylfuran	9.9	2	4.5	1	17.8	3
12	unknown	nd	1.5	nd	0.5	nd	–
13	3-octene-2-one	2.2	2	0.6	1	0.3	1
14	limonene	0.7	3	0.3	3	5.3	3
15	(*E*)-2-octenal	8.6	1	3.2	0.5	1.0	–
16	unknown	nd	2	nd	2	nd	2
17	nonanal	1.2	3	0.6	2	0.5	2
18	(*E*)-2-nonenal	0.2	2	nd	–	nd	–
19	unknown	nd	2	nd	–	nd	–
20	(*E*,*E*)-2,4-nonadienal + decanal	0.5	1	0.1	–	0.2	0.5
21	pentyl hexanoate	0.5	1.5	nd	0.5	nd	0.5

Identification or tentative identification of compounds according to Tab. 1.

Relative contents are expressed as arithmetic means of triplicates. Relative standard deviations were below 10 % for all compounds.

Odour intensities were obtained from 5 independent measurements. A seven-point scale ranging from 0 to 3 was used, and the value of ± 0.5 was considered as measurement deviation.

nd – not detected or under the quantitative threshold.

acid is dominant fatty acid in the seeds (71.8 % for Albín, 77.4 % for Gerlach and 73.3 % for Malsar), followed by oleic acid (15.5 % for Albín, 7.8 % for Gerlach and 11.8 % for Malsar), palmitic acid, stearic acid and several minor unsaturated fatty acids. Occurrence and relative contents of some odour-active volatiles detected in our study (Tab. 2) correspond well with these findings. Hexanal, the most abundant volatile compound in all analysed poppy seeds, is commonly monitored as a marker of lipid oxidation in vegetable oils [23], and represents a typical product of linoleic acid oxidation at 13-hydroperoxide [24–27]. Other aldehydes, such as pentanal, (*E*)-2-octenal and (*E*)-2-nonenal [25–27], and alcohols pentanol, hexanol and 1-octene-3-ol [25] were also previously reported to be volatiles produced by decomposition of linoleic acid hydroperoxides. Nonanal and decanal were identified as products of oleic acid autoxidation [24, 25], and (*E*)-2-hexenal as an oxidation derivative of linolenic acid [24, 25], which is also present in small quantities in poppy seeds [22]. Occurrence of 2-pentylfuran in vegetable oils was extensively studied in the past because of its bean-like flavour, which is undesirable in soybean oil, and subsequently mechanism of its formation from linoleic acid was described [25].

No pyrazine compound was detected in the volatile fractions of the analysed poppy seeds, in contrast to Guo et al. [13] who found 2,5-dimethylpyrazine, 2-ethyl-3-methylpyrazine and 2-ethyl-3,6-dimethylpyrazine to be responsible for the formation of typical roasted and nutty odour of full aroma poppy seed oil. They con-

cluded that these compounds are created during processing of poppy seeds prior to oil pressing as a consequence of denaturation and aminocarbonyl reactions. Similarly, KRIST et al. [7] observed pyrazines in an oil obtained from poppy seeds heated at 60 °C before pressing, and EMIR et al. [14] detected these compounds only in oils processing of which included seed roasting as a pre-treatment. Apparently, mild heating of ground poppy seeds during the sample preparation in our study was insufficient for the formation of pyrazines or, more likely, their potentially generated amounts did not exceed their olfactory detection thresholds.

Concerning relative content of individual compounds (Tab. 2), hexanal, hexanol, 2-pentylfuran, hexanoic acid and pentanol represented the most abundant volatiles in all three investigated varieties. In addition to them, pentanal and (*E*)-2-octenal were important in varieties Albín and Malsar. This is in good accordance with the study of KRIST et al. [7], in which the same compounds were found to be the most characteristic for different poppy oils. However, results obtained by GC-FID/O (Tab. 2) clearly demonstrated that not all of these volatiles provided also the highest odour intensities. This was most evident for the odourant hexanol, which showed a low intensity of 1, even though it was the second most abundant volatile compound. Similarly, hexanal, the most abundant compound in all varieties, showed only a moderate intensity of 2. In contrast, limonene showed the highest intensity of 3 in all varieties, and nonanal intensity of 3 in variety Albín and intensity of 2 in Gerlach and Malsar varieties, despite a low relative content. The special cases are the unidentified odour-active compounds, which had high olfactory intensities in some varieties, even though their relative concentrations were below detection thresholds of instrumental FID or MS detector. Therefore, from the perspective of their sensorial activity at given concentrations, limonene, nonanal, unknown compound No. 16, 2-pentylfuran, pentanol and hexanal were found to have the most significant impact on the aroma character and quality of all analysed poppy varieties. In addition to them, pentanal was found to be a further significant odour-active compound in varieties Albín and Malsar, and hexanoic acid in Albín and Gerlach volatile fractions.

Apparent discrepancies between the GC-MS or GC-FID and GC-FID/O results were apparently due to the specific features of GC-O, as threshold concentrations for olfactory detection of odour-active compounds often vary through several orders of magnitude, different psychophysical trends, selectivity of human nose and non-linear response for various kinds of odour-active compounds.

Concerning differences between individual analysed poppy varieties, the highest number of odour-active compounds was detected in the volatile fraction of white seed variety Albín, all of them contributing more or less significantly to its overall aroma. Total odour of this variety consisted of 23 volatile compounds (21 olfactory responses) with intensities ranging from 1 to 3. It is obvious that limonene and nonanal proved the highest odour intensities, and they are responsible mainly for fruity, slightly fatty and bitter tones. Moreover, nonanal appears to be a determining odour-active compound in the overall aroma, because of its odour that evoked ground poppy seeds at the given concentration. These dominant compounds were followed by the balanced mixture of eight volatiles with odour intensity of 2 characterized by a wide range of odour descriptions. To this group belonged pentanal, pentanol and hexanoic acid with pungent, yeasty and rancid odours, hexanal, (*E*)-2-nonenal and unknown compound No. 19 with fatty, waxy and nutty odours, as well as unknown compound No. 16 with balsamic and herbal odour, 3-octene-2-one providing bitter, tart-like odour with blueberry notes, and 2-pentylfuran possessing odour evoking green bean or mousy smell. 2-Pentylfuran was also described to have typical poppy seed odour at certain concentrations, indicating its importance for the formation of typical poppy seed aroma. Above mentioned impressions were supplemented with green, grassy, leafy and fruity notes attributed to 2-methyl-2-pentenal + unknown compound No. 2., (*E*)-2-hexenal, hexanol, (*E*)-2-octenal and pentyl hexanoate (odour intensities 1–1.5), as well as with another less intensive fatty, waxy, bitter and mushroom-like or paper-like odours, which were attributed to 3-methyl-1-butanol, (*E,E*)-2,4-nonadienal + decanal, unknown compound No. 9 and 1-octene-3-ol or unknown compound No. 12, respectively. Combination of all above mentioned components resulted in the unique, rich and well-balanced aroma of Albín variety, which eventually evoked more walnut than typical poppy seed aroma.

Volatile fractions of blue seed varieties Gerlach and Malsar consisted of comparable numbers of odour-active compounds (13 and 14, respectively). However, significant differences were observed in the odour intensities of individual volatiles. While in the odour profile of Malsar variety, limonene, pentanal, pentanol, hexanal, unknown compound No. 16 and nonanal were dominant (odour intensity 3 and 2), in the profile of Gerlach variety, 2-pentylfuran, limonene, pentanol, hexanoic

acid, unknown compound No. 16 and nonanal prevailed. Evident lower odour intensities were also recorded for some other compounds in the Gerlach volatile fraction, such as pentanal, 2-methyl-2-pentenal + unknown compound No. 2 and hexanal. Other volatiles, such as (*E*)-2-hexenal, (*E*)-2-octenal and unknown compound No. 12, missed completely in the Gerlach volatile fraction. As a consequence, Gerlach aroma lacked pleasant nutty, fatty or yeasty odours attributed to hexanal or pentanal, as well as fruity and green notes attributed to 2-methyl-2-pentenal + unknown compound No. 2., (*E*)-2-hexenal and (*E*)-2-octenal. On the contrary, bitter, metallic and rancid odours caused by hexanoic acid and 2-pentyl furan were intensified. In general, Malsar provided pleasant, full, more intensive, typical poppy seed aroma, whereas Gerlach had less-balanced aroma with predominant fatty, bitter and rancid notes.

Accepting the fact that both qualitative and quantitative aspects of nutritional as well as sensory properties of poppy plant products are strongly influenced by the origin, the growing, harvest and post-harvest conditions, they can vary considerably. Moreover, poppy seeds can be also subject to potential fraud and adulteration that lead to a poorer quality of these food commodities.

CONCLUSION

Comprehensive GC-FID/O and GC-MS analyses of the poppy seed volatile fractions obtained from three Slovakian varieties, namely, blue seed Malsar and Gerlach, and white seed Albín, proved in total the presence of 23 key odour-active compounds (21 olfactory responses), including nine aldehydes, four alcohols, one ketone, organic acid, furan derivative, ester, terpene and five unknown compounds. GC-MS data on relative contents of individual volatiles confirmed that hexanal, hexanol, 2-pentylfuran, hexanoic acid and pentanol belong to the most abundant poppy seed volatiles. Nevertheless, a special technique GC-FID/O revealed limonene, nonanal, unknown compound No. 16, 2-pentylfuran, pentanol and hexanal to be principal odour-active compounds in the volatile fractions of all three studied varieties. Comparison of obtained odour profiles showed significant differences between poppy seeds of investigated varieties in the total number, relative content and odour intensity of volatiles. Although volatile fractions of blue seed varieties consisted of comparable numbers of odour-active compounds, different odour intensities of pentanal, hexanal, hexanoic acid and 2-pentylfuran as well as of 2-methyl-2-pentenal + unknown compound No. 2., (*E*)-2-hexenal and (*E*)-2-octenal caused intensive, full, typical poppy seed odour of Malsar and, on the other hand, less-balanced odour of Gerlach variety with predominant bitter, fatty and rancid notes. White seed variety Albín was superior to both blue seed varieties in all investigated parameters and proved the richest, well-balanced and full odour, partially evoking walnut aroma. Considering also the high content of unsaturated fatty acids, Albín seems to be a beneficial variety with both excellent nutritional and sensorial quality, which predetermines it to various applications in the production of modern health-promoting foods.

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REFERENCES

1. Hlinková, A. – Havrlentová, M. – Šupová, J. – Bednárová, A.: Poppy seed (*Papaver somniferum* L.): effect of genotype and year of cultivation on variability in its lipid composition. Journal of Microbiology, Biotechnology and Food Sciences, *1*, 2012, pp. 908–922. ISSN: 1338-5178.
2. Fejér, J.: Breeding aspects of poppy in Slovakia. Acta Horticulturae, *1036*, 2011, pp. 101–105. DOI: 10.17660/ActaHortic.2014.1036.10.
3. Fejér, J. – Salamon, I.: Poppy (*Papaver somniferum* L.) as a special crop in the Slovakian history and culture. Acta Horticulturae, *1036*, 2014, pp. 107–109. DOI: 10.17660/ActaHortic.2014.1036.11.
4. FAOSTAT [online]. Rome : Food and Agriculture Organization of the United Nations, 2015 [cit. 26 January 2016]. <http://faostat3.fao.org/download/Q/QC/E>.
5. Özcan, M. M. – Atalay, Ç.: Determination of seed and oil properties of some poppy (*Papaver somniferum* L.) varieties. Grasas Aceites, *57*, 2006, pp. 169–174. DOI: 10.3989/gya.2006.v57.i2.33.
6. Erinç, D. D. – Tekin, A. – Özcan, M. M.: Determination of fatty acid, tocopherol and phytosterol contents of the oils of various poppy (*Papaver somniferum* L.) seeds. Grasas Aceites, *60*, 2009, pp. 375–381. DOI: 10.3989/gya.129508.
7. Krist, S. – Stuebinger, G. – Unterweger, H. –

Bandion, F. – Buchbauer, G.: Analysis of volatile compounds and triglycerides of seed oils extracted from different poppy varieties (*Papaver somniferum* L.). Journal of Agricultural and Food Chemistry, *53*, 2005, pp. 8310–8316. DOI: 10.1021/jf0580869.

8. Bozan, B. – Temelli, F.: Extraction of poppy seed oil using supercritical CO_2. Journal of Food Science, *68*, 2003, pp. 422–426. DOI: 10.1111/j.1365-2621.2003.tb05688.x.
9. Bozan, B. – Temelli, F.: Chemical composition and oxidative stability of flax, safflower and poppy seed and seed oils. Bioresource Technology, *99*, 2008, pp. 6354–6359. DOI: 10.1016/j.biortech.2007.12.009.
10. Czaplicki, S. – Ogrodowska, D. – Derewiaka, D. – Tańska, M. – Zadernowski, R.: Bioactive compounds in unsaponifiable fraction of oils from unconventional sources. European Journal of Lipid Science and Technology, *113*, 2011, pp. 1456–1464. DOI: 10.1002/ejlt.201000410.
11. Li, C. – Zhao, X. – Chen, N. – Chen, Y.: Chemical composition of the volatile oil of *Papaver somniferum*. Journal of Lanzhou University, *26*, 1990, pp. 145–146. ISSN: 0455-2059. In Chinese.
12. Krist, S. – Stuebinger, G. – Bail, S. – Unterweger, H.: Detection of adulteration of poppy seed oil with sunflower oil based on volatiles and triacylglycerol composition. Journal of Agricultural and Food Chemistry, *54*, 2006, pp. 6385–6389. DOI: 10.1021/jf060500x.
13. Guo, J. – Kong, D. – Hu, L.: Comparative analysis of volatile flavour compounds of poppy seed oil extracted by two different methods via gas chromatography/mass spectrometry. Journal of PharmaSciTech, *4*, 2015, pp. 36–38. ISSN: 2231-3788. <http://www.pharmascitech.in/admin/php/uploads/87_pdf.pdf>
14. Emir, D. D. – Güneşer, O. – Yilmaz, E.: Cold pressed poppy seed oils: sensory properties, aromatic profiles and consumer preferences. Grasas Aceites, *65*, 2014. DOI: 10.3989/gya.109213.
15. Delahunty, C. M. – Eyres, G. – Dufour, J.-P.: Gas chromatography-olfactometry. Journal of Separation Science, *29*, 2006, pp. 2107–2125. DOI: 10.1002/jssc.200500509.
16. d'Acampora Zellner, B. – Dugo, P. – Dugo, G. – Mondello, L.: Gas chromatography-olfactometry in food flavour analysis. Journal of Chromatography A, *1186*, 2008, pp. 123–143. DOI: 10.1016/j.chroma.2007.09.006.
17. Janáčová, A. – Sádecká, J. – Kohajdová, Z. – Špánik, I.: The identification of aroma-active compounds in Slovak brandies using GC-sniffing, GC-MS and sensory evaluation. Chromatographia, *67*, 2008, pp. S113–S121. DOI: 10.1365/s10337-008-0635-1.
18. van Den Dool, H. – Kratz, P. D.: A generalization of the retention index system including gas-liquid partition chromatography. Journal of Chromatography A, *11*, 1963, pp. 463–471. DOI: 10.1016/S0021-9673(01)80947-X.
19. Belajová, E. – Suhaj, M.: Compositional profiling of Slovakian wines from distinct production systems by analysis of main saccharides and glycerol. Journal of Food and Nutrition Research, *51*, 2012, pp. 173–183. ISSN: 1336-8672 (print), 1338-4260 (online). <http://www.vup.sk/download.php?bulID=1309>
20. Koreňovská, M. – Suhaj, M.: Assessment of some European goats' cheeses by multivariate elemental data comparison. Journal of Food and Nutrition Research, *49*, 2010, pp. 94–103. ISSN: 1336-8672 (print), 1338-4260 (online). <http://www.vup.sk/download.php?bulID=128>
21. Sádecká, J. – Polovka, M. – Kolek, E. – Belajová, E. – Tobolková, B. – Daško, L. – Durec, J.: Orange juice with pulp: impact of pasteurization and storage on flavour, polyphenols, ascorbic acid and antioxidant activity. Journal of Food and Nutrition Research, *53*, 2014, pp. 371–388. ISSN: 1336-8672 (print), 1338-4260 (online). <http://www.vup.sk/download.php?bulID=1626>
22. Hlinková, A. – Čertík, M. – Havrlentová, M.: Investigation of lipid content and fatty acids composition in selected poppy cultivars (*Papaver somniferum* L.). Agriculture, *57*, 2011, pp. 118–123. ISSN: 1338-4376. <http://www.agriculture.sk/fileadmin/files/2011/Issue_3/Hlinkova.pdf>
23. Ma, C. – Ji, J. – Tan, C. – Chen, D. – Luo, F. – Wang, Y. – Chen, X.: Headspace solid-phase microextraction coupled to gas chromatography for the analysis of aldehydes in edible oils. Talanta, *120*, 2014, pp. 94–99. DOI: 10.1016/j.talanta.2013.11.021.
24. Lee, J. – Kim, D.-H. – Chang, P.-S. – Lee, J.: Headspace-solid phase microextraction (HS-SPME) analysis of oxidized volatiles from free fatty acids (FFA) and application for measuring hydrogen donating antioxidant activity. Food Chemistry, *105*, 2007, pp. 414–420. DOI: 10.1016/j.foodchem.2006.12.059.
25. Choe, E. – Min, D. B.: Mechanisms and factors for edible oil oxidation. Comprehensive Reviews in Food Science and Food Safety, *5*, 2006, pp. 169–186. DOI: 10.1111/j.1541-4337.2006.00009.x.
26. García-Martínez, M. C. – Márquez-Ruiz, G. – Fontecha, J. – Gordon, M. H.: Volatile oxidation compounds in a conjugated linoleic acid-rich oil. Food Chemistry, *113*, 2009, pp. 926–931. DOI: 10.1016/j.foodchem.2008.08.020.
27. Ivanova-Petropulos, V. – Mitrev, S. – Stafilov, T. – Markova, N. – Leitner, E. – Lankmayr, E. – Siegmund, B.: Characterisation of traditional Macedonian edible oils by their fatty acid composition and their volatile compounds. Food Research International, *77*, 2015, pp. 506–514. DOI: 10.1016/j.foodres.2015.08.014.

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