Headspace Solid-Phase Microextraction Use for the Characterization of Volatile Compounds in Vegetable Oils of Different Sensory Quality

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Headspace solid-phase microextraction (HS-SPME) was used to isolate the volatile compounds, which are formed during peroxidation of fatty acids in vegetable oils. Isolated compounds were characterized by GC−MS and quantified using GC with FID detection. Four fibers for HS-SPME method development were tested, and the divinylbenzene/carboxene/PDMS fiber was selected as providing the best detection of analyzed compounds. Extraction curves, limits of detection, repeatability, and linearity were investigated for 14 aldehydes, ketones, hydrocarbons, and alcohols being products of fatty acids autoxidation. Limits of detection for 11 of these were below 1 μg/L. For quantitative purposes, to minimize the influence of temperature on hydroperoxide formation and the changes in the volatiles profile of the extracts, sampling was performed at 20 °C. For compound characterization by GC−MS, sampling temperature of 50 °C was applied. The developed method was applied to the analysis of refined and cold-pressed rapeseed oil stored at 60 °C for 10 days, and for 10 different vegetable oils of various degree of peroxidation. All samples were subjected to sensory analysis. The results of PCA sensory analysis were related to the amount of volatile compounds isolated by SPME method. In cases where the amount of compounds was highest, the samples were perceived as the worst, whereas those with low levels of volatile compounds were the most desired ones according to sensory evaluation. The relation was observed for both total volatiles, quantified C5−C9 aldehydes, and 14 compounds selected in method development. SPME revealed to be a rapid and sensitive method for the extraction and quantitation of trace volatile compounds from plant oils even at ambient temperature.

Keywords: *Solid-phase microextraction; SPME; lipids peroxidation; volatile compounds; vegetable oils; fats*

INTRODUCTION

Major components of vegetable oils are fatty acids, both saturated and unsaturated, mainly bound to glycerol as triacylglycerols. The allyl groups in unsaturated fatty acids are highly susceptible to free-radical reactions. In the presence of oxygen, unsaturated fatty acids undergo decomposition even at low temperatures. The saturated fatty acids are relatively stable to oxidation at temperatures of <60 °C, but they oxidize at higher temperatures. As a result of autoxidation, initially odorless monohydroperoxides are formed, which eventually break down into mainly volatile products. This group comprises aldehydes, ketones, alcohols, acids, hydrocarbons, furanones, and lactones (Grosch, 1982). Due to the low odor thresholds of the majority of these compounds, the presence of volatile hydroperoxide degradation products even at low concentration impairs the sensory properties of oils or fat-containing product. As the odor thresholds of aliphatic aldehydes in oils can be as low as 0.0015 μg/L (2,6-*trans*,*cis*-nonadienal) or 0.0001 μg/L for 1-octene-3-one (Grosch, 1982), there is a need for an accurate quantitation of volatiles in fat-containing foods.

Methods applied to the analysis of volatiles resulting from fat degradation involve static headspace, dynamic headspace, and direct chromatography (placing vegetable oil into the injection port of gas chromatograph) and have been used for several decades (Dupuy, 1973; Bassette and Ward, 1975; Warner and Nelson, 1996; Ulbreth and Roubicek, 1993). Solid-phase microextraction (SPME)—a solventless method for the isolation of volatile compounds—was developed in the early 1990s (Arthur and Pawliszyn, 1990; Zhang and Pawliszyn, 1993). Since that time, it has been applied to analysis of environmental contaminants and also food flavor compounds. However, data in the literature on the application of SPME to analysis of vegetable oil volatiles or lipid degradation volatiles is rare (Keszler et al., 1998).

Our work was aimed at the development of a SPME sampling procedure for qualitative and quantitative determination of volatile compounds present in edible oils. The goal was also to apply the developed SPME method for determination of changes in oil volatiles during storage at elevated temperature and their correlation with sensory characteristics of investigated samples.

EXPERIMENTAL PROCEDURES

Materials. The following 14 compounds were used for the method evaluation: *n*-pentane, 2-butanone, *n*-butanol, pentanal, pentanol, hexanal, 2-heptanone, heptanal, 2-heptenal, 1-octen-3-ol, octanal, 2-nonanone, nonanal, *trans*, *trans*-2,4-

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decadienal. They are known to appear in the process of lipids oxidation. Compounds were of the highest purity available (usually >99%), purchased from the Sigma-Aldrich-Fluka company. Standards of compounds used for the peak identity confirmation in GC−MS experiment originated mainly from Aldrich and also from Bedoukian Res. Corp., J. T. Baker, Pfaltz & Baur Inc., and Theta Corp. For method evaluation, four types of fibers, 85 μm polyacrylate (PA), 100 μm poly(dimethylsiloxane) (PDMS), carbowax/divinylbenzene (CW/DVB), and divinylbenzene/carboxene in poly(dimethylsiloxane) (DVB/CAR/PDMS) (all Supelco Inc., Bellefonte, PA), were chosen. All fibers were preconditioned in the gas chromatograph injection port at the temperature and for the period of time recommended by producer.

The developed SPME isolation method was applied in two experiments: in accelerated storage test (60 °C) of rapeseed oil and in comparison of different oils kept at room temperature.

For the accelerated storage test, cold-pressed and refined rapeseed oils purchased at a local store were used. A total of 200 mL of each oil was put into a 1000 mL flat-bottom flask in triplicates, and the capped flasks were placed in a thermostat at 60 °C. First flask was taken out for the thermostat after 3 days, next one after 5 days, and the final one after 10 days of storage. All samples were stored at −20 °C until day 10 and then analyzed. Peroxide values (PV) of refined and cold-pressed rapeseed oils were 3.4 and 2.0, respectively, at the beginning of the experiment. After 3 days, the values were 46.0 and 26.5, respectively, after 5 days 57.2 and 31.9, and after 10 days 91.3 and 36.1.

For the comparison of different oils, the following were used: peanut oil (PV = 1.2), soya I (PV = 1.7), soya II (PV = 4.5), rapeseed/soya mixture (PV = 20), rapeseed refined (PV = 5.3), rapeseed cold-pressed (PV = 19), linseed (PV = 18), sunflower (PV = 4.8), corn (PV = 1.1), and olive oil (PV = 10.1). Linseed oil was received as a gift from the Polcargo company, and the remaining nine oils were purchased at the local supermarket. Five oils were fresh according to the labels notice (peanut oil, corn oil, sunflower, rapeseed, soya I) at the time of analysis. The remaining samples were analyzed after prolonged storage, several months beyond the "best before" date shown on the label. Oil samples were stored in darkness at room temperature.

Sample Preparation and Analysis. Standards were weighed into a 100 mL volumetric flask (approximately 0.100 g each volatile) to obtain standard stock solution of around 1 mg/mL each, and the flask was filled with freshly refined rapeseed oil. The stock solution was then diluted 10-fold using the same oil. This solution was used to prepare standard curves. Standards of the compounds analyzed were dissolved directly in oil to eliminate the influence of the solvent presence in very high concentration compared to standards, on their adsorption on the fiber. Both standard solutions and oil samples were analyzed in the same way: 10 mL of oil sample or oil spiked with standards was placed in a 20 mL headspace vial and kept at 20 °C. The Teflon-lined septum covering the vial was pierced with a SPME needle and the fiber was exposed to the oil headspace for 30 min. The fiber was then retracted into the needle and immediately transferred to the gas chromatograph. The fiber was desorbed for 5 min in the gas chromatograph injection port. For the SPME analyses, Hewlett-Packard HP 5890II coupled to 5971 MSD quadrupole mass spectrometer and HP 6890 Plus gas chromatograph with FID detector were used. Compounds were resolved on a MDN-5 and HP-5 fused silica columns (30 m × 0.25 mm × 0.25 μm, Supelco, and 30 m × 0.32 mm × 0.25 μm, Hewlett-Packard, respectively) under the following conditions: injection port temperature, 270 °C (for CAR/DVB/PDMS fiber); helium flow, 0.6 mL/min (HP 5890) and 2 mL/min (HP 6890); oven temperature, 40 °C (3 min) then 4 °C/min to 100 and 15 °C/min to 220 °C (5 min). All samples were run at least in triplicates. To compare samples analyzed, the contents of selected compounds in parts per billion, or peak areas of total ion chromatograms (TIC), were used. Identity of compounds in qualitative analyses was verified using standards, or in some cases only tentative identification by NIST mass spectra library search was performed.

Table 1. Vocabulary of Oil Odor Descriptors

attribute	description
1. acidic	associated with acid, or fermented products
2. metallic	reminiscent rust (water with nails kept in)
3. sweet	associated with maltol or sugar-wool
4. green	characteristic for freshly cut grass or leaves
5. oxidized	characteristic for rancid oil
6. musty	reminiscent an old basement
7. earthy	characteristic for raw potato or raw beetroot
8. desirability	general desirability of sample odor

Descriptive Analysis of Oil Samples. The sensory analysis was performed by a 10 member panel, experienced in descriptive analysis. The odor profiling analysis of all samples was run in triplicates (three sessions) proceeded by an introductory session. Seven odor attributes (developed in earlier study) and general desirability of sample's odor (Table 1) were scaled on linear 10 cm scales anchored on both sides for the intensity of attributes as "none" and "very strong" and for desirability as "not desired" and "very desired". The odor attributes were chosen according to the "Basic Flavor Descriptive Language" from Givaudan Roure Flavor Ltd. (Stampanoni, 1998). The 20 g samples of oil were presented to the panelists in 100 mL closed vessels, preheated to 40 °C prior to the analysis. The results from the linear scales were converted into numerical values for data analysis. Mean, variance, and standard deviations were calculated for all attributes of each sample, for each session separately, and across all three sessions. The obtained data were counted from 30 replicates and after statistical interpretation by multivariate procedure presented as graphic projection of Principal Component Analysis (PCA) (Baryłko-Pikielna et al., 1992). Descriptors used in sensory analysis have been presented in Table 1.

RESULTS

First, the optimal parameters for the isolation and separation of volatile compound standards spiked into rapeseed oil were established. Then, the SPME method was applied to the characterization of volatiles of two rapeseed oils subjected to accelerated oxidation at 60 °C and quantitation of some of volatile compounds in 10 samples of oils. The results of the analysis of volatile compounds were compared with sensory studies results.

SPME Method Development. *Fiber Choice.* Four fiber types were evaluated to choose the one able to absorb the greatest amount (expressed as peak areas) of compounds at 20° C during 30 min extraction. A standard mixture of 14 compounds in oil at a concentration of 10 μg/L each was used. Each extraction was repeated five times. Of all the fibers evaluated PA had the lowest ability to extract compounds, only 6 being detected under the conditions described above. PDMS also showed little extraction capacities. Fiber coated with CW/DVB allowed all compounds to be detected, as did DVB/CAR/PDMS fiber, but the latter provided much larger peak areas (Figure 1), and therefore, DVB/CAR/PDMS was chosen for subsequent analyses. Extra peaks observed on chromatograms originated from rapeseed oil used as a matrix and few of them from column bleed.

Fiber Exposure Time. Solutions of standards containing 10 μg/L of each compound in oil were prepared, and DVB/CAR/PDMS fiber was exposed in different vials with standards mixture for 1, 5, 10, 30, 60, and 90 min, respectively. Each determination was repeated four times. The rate of extraction was highest during the first 10 min of fiber exposure (Figure 2). After this time, the rate decreased, and for some compounds (nonanal,

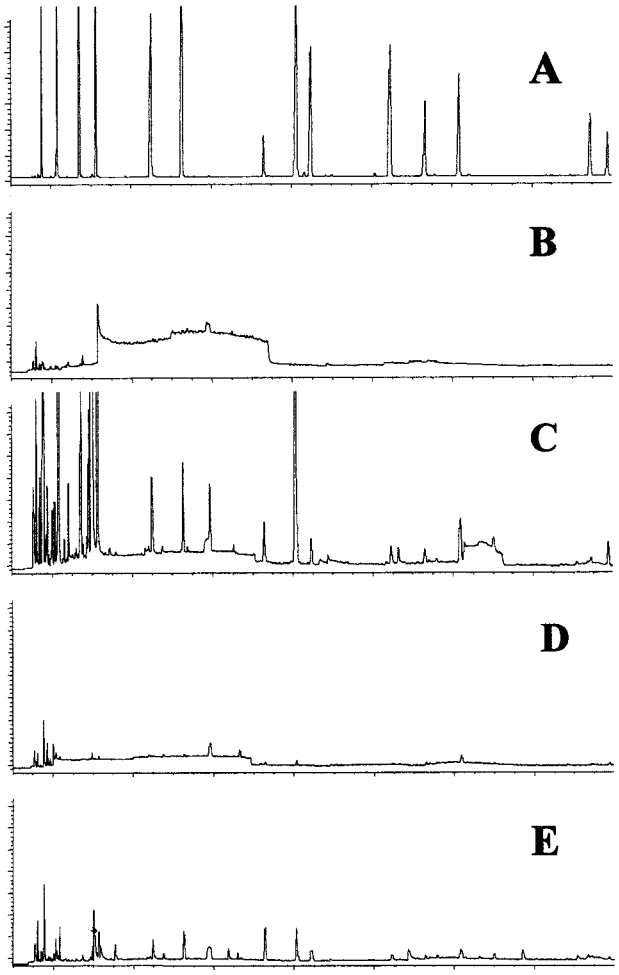

Figure 1. Chromatogram of freshly refined rapeseed oil spiked with 10 μg/L of each standard compounds obtained using various fibers (B−E); (A) chromatogram of standards at 10 mg/L; (B) PA fiber; (C) DVB/CAR/PDMS fiber; (D) PDMS fiber; (E) CW/DVB fiber. For the clarity of presentation decadienal peak eluting as the last has not been shown. The intensity scale in chromatograms B−E is the same.

2-nonanone), equilibrium seemed to have been established, whereas for most compounds, peak areas increased during the whole course of the extraction, indicating that equilibrium had not been reached within 90 min. Taking into consideration that chromatographic analysis took just over 30 min, an extraction time of 30 min was chosen. This ensured sufficiently low limits of detection without extending total analysis time.

Limits of Detection, Repeatability, and Calibration Curves. Limits of detection (LOD) can be defined as three times the standard deviation obtained for an analyte concentration no higher than 10 times the method detection limit (Pawliszyn, 1997). In our experiments, LODs were estimated using this definition. Limits of detection and data on precision are shown in Table 2. The limits of detection ranged from 0.04 μg/L for hexanal to 2.24 μg/L for 2,4-decadienal, 11 of the 14 compounds being detectable below 1 μg/L when flame ionization detector (FID) was used. Repeatability expressed as relative standard deviation (RSD) for peak areas at 10 μg/L standards concentration was generally below 7.5%; only three compounds, heptanal, nonanal, and 2,4-decadienal, were characterized by RSD values higher than 10%. For standards concentration at 10 mg/L, RSD values ranged between 0.78 and 5.92%. For the

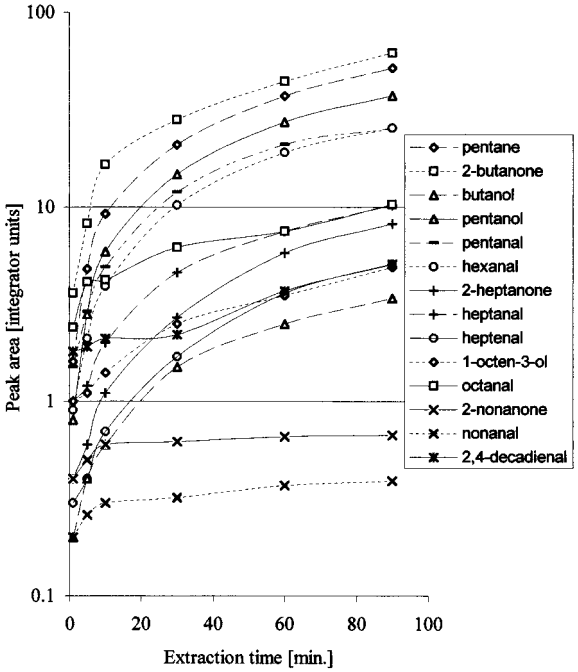

Figure 2. SPME extraction curves of standard mixture at 10 μg/L. Extraction was performed at 20 °C using DVB/CAR/PDMS fiber.

determination of compounds in various oils, calibration curves have been prepared in the range of 8−10 μg/L to 1 mg/L. Linearity of these curves was characterized by r^2 values of 0.918 (pentanal) to 0.998 (pentanol, heptanal).

Characterization of Volatile Compounds in Rapeseed Oil by SPME-GC−MS. Gas chromatography with mass spectrometry was used for the identification of volatile compounds extracted from rapeseed oil using SPME fiber. Coupling mass spectrometer to gas chromatograph as a detector enables the reliable identification of majority of compounds. However, LOD obtained using a mass spectrometer working in scan mode are usually much higher than that using FID. They will depend, to a great extent, besides the molecule nature, on the mass detector conditions—scan range, ion source conditions, and quality of instrument—and therefore will differ from instrument to instrument. Because the TIC chromatograms of oils volatiles acquired at sampling temperature of 20 °C exhibited many peaks of poor intensity (Figure 3A), for the qualitative characterization of volatile compounds profile in stored oils, extraction at 50 °C was chosen (Figure 3B).

In Table 3, volatile compounds identified by SPME-GC−MS method in rapeseed oils stored for 10 days at 60 °C have been shown. Of 56 compounds, most were detected in both cold-pressed and refined rapeseed oils. Of volatile compounds, 21 aldehydes, 14 of them unsaturated, prevailed. Also acetic, propanoic, hexanoic, and nonanoic acids were detected. Their characteristic triangular peaks on nonpolar columns elute for a relatively long time, coeluting with other compounds. Contrary to solvent extracts analysis, acids cannot be neutralized, when SPME is used, which would eliminate their peaks from chromatogram facilitating identification of coeluting compounds. When samples subjected to accelerated test for 10 days were extracted at 20 °C, it was possible to obtain reliable spectra for most compounds. For some of them (octane, 2-pentenal, 2-butenal, coeluting pentanal, and 2-ethyl furan), peak areas

Table 2. Limits of Detection (LOD), Repeatability (as RSD), and Contents in Two Examined Oils of Compounds Used in Method Development

compd	retention time (min)	LOD (μg/L)	RSD (%) 10 μg/L	RSD (%) 10 mg/L	linseed oil[d]	sunflower oil[e]
pentane	1.780	1.46	2.84	1.14	211.7[a]	42.2[a]
2-butanone	2.168	0.06	2.23	1.71	39.6[a]	Nd[f]
butanol	2.723	0.13	7.32	0.83	56.8[a]	8.7[b]
pentanal	3.129	0.35	3.18	0.78	11.1[b]	9.8[b]
pentanol	4.499	0.06	4.37	1.89	10.1[b]	Tr[g]
hexanal	5.257	0.04	3.91	3.20	23.5[a]	1.1[b]
2-heptanone	8.121	0.21	5.41	3.18	19.7[a]	Tr
heptanal	8.464	0.59	12.55	3.26	2.9[b]	Tr
2-heptenal	10.457	0.08	5.69	4.89	15.0[a]	2.9[b]
1-octen-3-ol	11.341	0.61	5.49	4.47	8.0[a]	Nd
octanal	12.171	1.39	7.49	5.92	19.8[a]	11.1[c]
2-nonanone	15.489	0.26	5.85	4.94	12.9[a]	0.7[c]
nonanal	15.903	0.26	11.39	5.59	13.9[a]	2.9[c]
decadienal	21.175	2.24	10.87	5.36	17.7[a]	24.1[b]

[a] RSD < 5%. [b] RSD 5−15%. [c] RSD > 15%; $n = 3$. [d] Linseed oil of inferior sensory quality. [e] Good quality sunflower oil. [f] Nd, not detected. [g] Tr, traces.

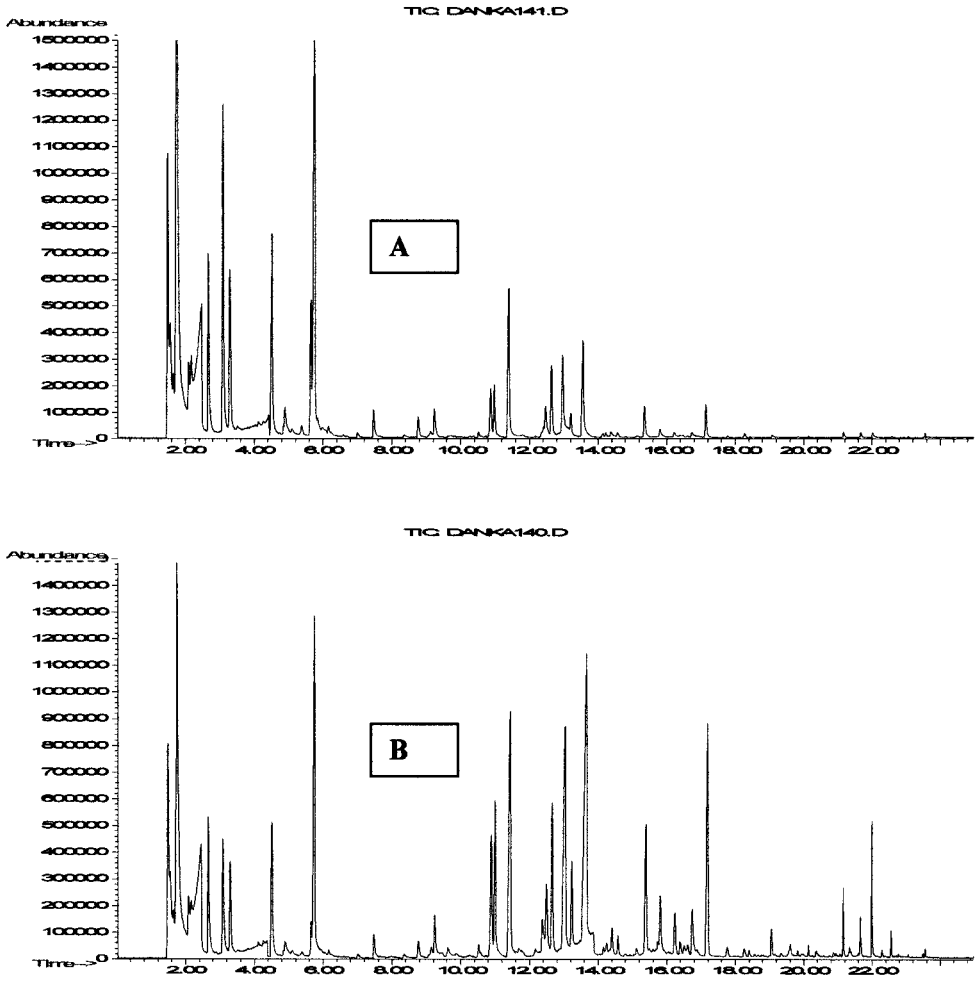

Figure 3. TIC chromatograms of volatile compounds extracted with DVB/CAR/PDMS fiber from cold-pressed rapeseed oil, stored at 60 °C for 10 days. (A) TIC chromatogram of compounds extracted at 20 °C; (B) TIC chromatogram of compounds extracted at 50 °C.

after extraction at 20 °C were larger compared to extraction at 50 °C. However, extraction of compounds boiling at higher temperatures was much less effective at 20 °C, which can be seen on the chromatograms A and B (Figure 3). Therefore, for qualitative purposes, when mass spectrometer is used as detector, extraction at 50 °C is recommended, especially for fresh oils, containing low amount of volatiles, but one has to remember that for quantitative analysis prolonged heating even at 50 °C can accelerate labile compounds degradation.

Changes of Volatile Compounds during Accelerated Storage of Rapeseed Oil. Samples of cold-pressed and refined rapeseed oil after 0, 3, 5, and 10 days storage at 60 °C were subjected to SPME extraction at 20 and 50 °C. Figure 4 shows total peak areas

Table 3. Major Volatile Compounds Isolated from Refined and Cold-Pressed Rapeseed Oil Using SPME at 50 °C[a]

T_r (min)	compd	refined	cold-pressed
1.75	pentane[b]	+	+
2.16	butanal[b]	+	+
2.45	acetic acid[b]	+	+
2.66	2-butenal[b]	+	+
3.08	unidentified	+	+
3.30	2-ethyl furan[b]/pentanal[b]	+	+
4.37	propanoic acid	+	+
4.51	2-pentenal[b]	+	+
4.98	pentanol	+	−
5.65	octane[b]	+	+
5.75	hexanal[b]	+	+
6.45	unidentified	+	−
7.01	2,4-dimethyl 2-pentene	+	+
7.48	2-hexenal[b]	+	+
8.77	2-heptanone[b]	+	+
9.25	heptanal[b]	+	+
9.63	2,4-hexadienal	+	+
10.89	unidentified	+	+
11.00	unidentified	+	+
11.44	2-heptenal[b]	+	+
12.38	1-octene-3-ol[b]	+	+
12.50	6-methyl-5-hepten-2-one	+	+
12.67	2-pentyl furane[b]	+	+
12.88	6-methyl-5-hepten-2-ol	+	+
13.05	2,4-heptadienal	+	+
13.24	octanal[b]	+	+
13.66	2,4-heptadienal	+	+
14.41	unidentified	+	+
14.15	limonene	−	+
14.56	3-octen-2-one	+	+
14.86	hexanoic acid[b]	+	+
15.12	2(3h) 5-ethyl dihydrofuranone	+	+
15.39	2-octenal	+	+
15.82	unidentified	+	+
15.96	1-octanol	−	+
16.10	unidentified	+	−
16.30	unidentified	+	−
16.61	2-nonanone[b]	−	+
16.74	3,5-octadien-2-one	+	+
17.19	nonanal[b]	+	+
17.76	unknown	+	+
18.38	3-nonen-2-one	+	−
19.06	2-nonenal[b]	+	+
19.61	unidentified	+	−
19.71	unidentified	+	+
19.82	2-decanone[b]	+	+
19.96	unidentified	+	−
20.14	decanal	+	+
20.34	2,4-nonadienal	+	−
20.87	unidentified	+	+
21.15	2-decenal[b]	+	+
21.34	nonanoic acid	+	+
21.65	2,4-decadienal	+	+
21.99	2,4-decadienal[b]	+	+
22.28	unidentified	+	+
22.56	2-undecenal[b]	+	+

[a] Oils were stored in darkness, at 60 °C for 10 days. [b] Identity of compounds confirmed by GC−MS analysis of standards, rest of compounds tentatively identified based on NIST mass spectra library search.

of compounds extracted from cold-pressed oil using SPME. Total amount of volatile compounds increased during storage from 6.9 and 25.6 million integrator units to 396.3 and 583.1 million for 20 and 50 °C sampling temperatures, respectively. The same rule was observed for volatiles extracted from refined oil (Figure 5). Total amount of volatiles expressed in peak areas was initially lower for refined than for cold-pressed oil (2.9 and 4.7 million for temperatures 20 and 50 °C, respectively). However, after 3, 5, and 10 days of storage, the amount

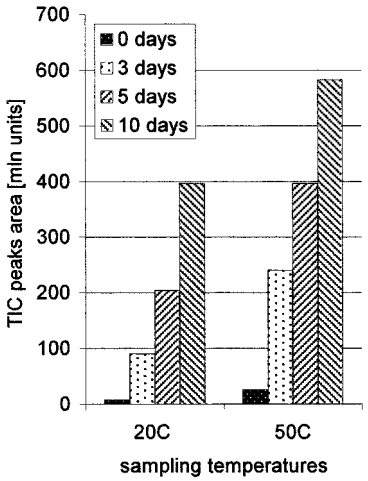

Figure 4. Total volatile compounds (expressed as peaks area in intergator units) of cold-pressed rapeseed oil sampled after 0, 3, 5, and 10 days of storage at 60 °C. Volatiles were sampled using DVB/CAR/PDMS fiber at 20 and 50 °C for 30 min. mln = million.

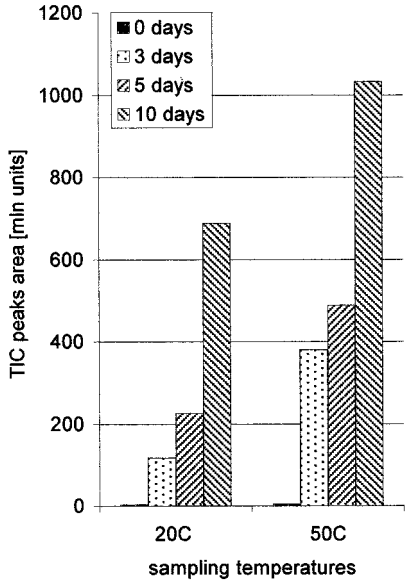

Figure 5. Total volatile compounds (expressed as peaks area in intergator units) of refined rapeseed oil sampled after 0, 3, 5, and 10 days of storage at 60 °C. Volatiles were sampled using DVB/CAR/PDMS fiber at 20 and 50 °C for 30 min. mln = million.

of volatiles from refined oil was higher than from cold-pressed one. The difference was most significant after 10 days.

During storage experiment, apart from total volatiles expressed as peak areas, the contents of major aldehydes were measured using GC with FID detection. The SPME extraction was performed at 20 °C, and the following aldehydes were quantified: pentanal, hexanal, 2-hexenal, heptanal, 2-heptenal, octanal, and nonanal. For these compounds, a rapid increase in their contents was noted. Initially, the aldehyde contents for cold-pressed rapeseed oil was 436.2 μg/L and for rapeseed refined was 149.1 μg/L. Similar to the total volatile compounds, the concentration of investigated aldehydes increased during storage, and also after 10 days it was much higher for the refined oil than for the cold-pressed one (Figure 6). Total aldehydes contents for cold-pressed oil neared 22 mg/L, whereas for refined it was almost

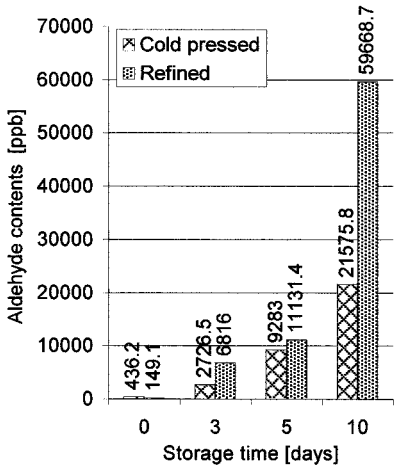

Figure 6. Contents of volatile aldehydes in refined and cold-pressed rapeseed oils extracted using SPME method at 20 °C. Total aldehydes comprise pentanal, hexanal, 2-hexenal, heptanal, 2-heptenal, octanal, and nonanal.

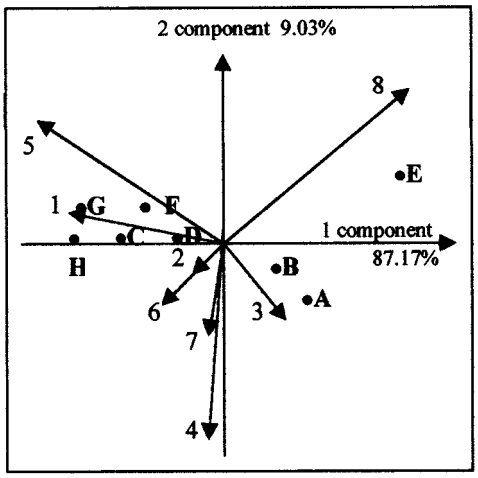

Figure 7. PCA plot of sensory profile data of eight rapeseed oil samples from the storage experiment. Sample codes: (A) cold-pressed, fresh; (B) cold-pressed, 3 days at 60 °C; (C) cold-pressed, 5 days at 60 °C; (D) cold-pressed, 10 days at 60 °C; (E) refined, fresh; (F) refined 3 days at 60 °C, (G) refined 5 days at 60 °C; (H) refined 10 days at 60 °C. Descriptors: (1) acidic; (2) metallic; (3) sweet; (4) green; (5) oxidized; (6) musty; (7) earthy; (8) desirability.

60 mg/L. In cold-pressed oil, hexanal was the most abundant aldehyde in fresh oil and after 3 and 5 days of storage. After 10 days, 2-heptenal was the most abundant of analyzed aldehydes (7.4 mg/L compared to 6.8 mg/L for hexanal and 4.3 mg/L for nonanal). The contents of hexanal and 2-heptenal were similar in fresh refined rapeseed oil, but after only 3 days, the amount of 2-hexenal was over twice the amount of hexanal and reached after 10 days, 45 mg/L. Next two aldehydes were nonanal (4.3 mg/L) and hexanal (4.2 mg/L).

To classify the oil samples according to their sensory quality, all the samples from the storage experiment were coded with letters A−H and subjected to descriptive analysis of oil odors. Data obtained after statistical interpretation by PCA are presented in Figure 7. All analyzed oil samples were compared with each other in relation to all used odor attributes. In this case, sensory analysis revealed significant differentiation in odor profiles of analyzed samples: the main odor descriptors responsible for the differentiation were oxidized (5), green (4), acidic (1), and desirability (8).

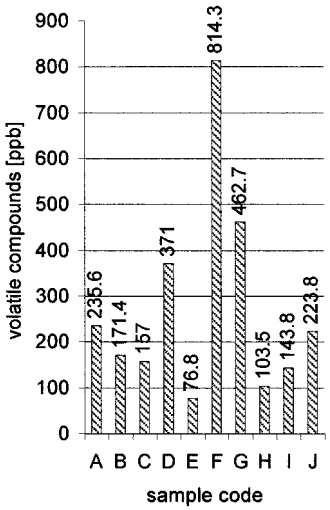

Figure 8. Contents of selected volatile compounds (see Table 2) in various oils extracted using SPME method at 20 °C. Sample codes: (A) peanut, fresh; (B) soy I, fresh; (C) soy II, stored; (D) rapeseed/soy, stored; (E) rapeseed refined, fresh; (F) rapeseed cold-pressed, stored; (G) linseed, stored; (H) sunflower, fresh; (I) corn, fresh; (J) olive oil, stored.

The most desired and odorless was sample E (refined, fresh). Samples A and B (cold-pressed fresh and after 3 days of storage) were of very similar, moderately desired odor: sweet (3) and green (4). All the remaining samples, C, D (cold-pressed after 5 and 10 days at 60 °C), and F, G, and H (refined after 3, 5, and 10 days at 60 °C) characterized undesired sensory quality with oxidized (5), acidic (1), and musty (6) off-odors. The worst one was the sample H (refined, after 10 days of storage). From the sensory evaluation, it can be concluded that storage at elevated temperature produced undesirable changes of oil odor in all examined samples, excluding B, already after 3 days.

Analysis of selected volatile compounds in various vegetable oils by HS SPME. Volatile compounds chosen for method evaluation were determined in 10 oil samples which varied in terms of "freshness" expressed in peroxide value and fatty acids composition. Quantified compounds represented aldehydes, but also alcohols, ketones, and hydrocarbons. Choice of compounds was determined by their diversified chemical nature helpful in SPME method development, not by the results of GC−MS findings of examined rapeseed oils. On Figure 8, a total amount of 14 compounds quantified using the elaborated SPME method has been shown. Of the quantified compounds, in almost all samples pentane prevailed. The exceptions were fresh rapeseed and corn oils. Other compounds present in a relatively high concentration were pentanal, hexanal, and 2,4-decadienal. Minor compounds among the quantified ones were pentanol, heptanal, and 2-nonanone. When fresh oils were compared with the stored ones, for the first group the sum of analyzed compounds was generally lower than 200 μg/L. On the other hand, oils which were stored for longer time had more quantified volatiles. Olive, rapeseed/soy, linen, and cold-pressed rapeseed oils contained up to 814.3 μg/L of quantified volatile compounds. Only soy II oil (characterized by relatively low PV value 4.5) after prolonged storage at room temperature was characterized with the sum of quantified volatiles lower than 200 μg/L. The total peak areas paralleled the quantitation findings.

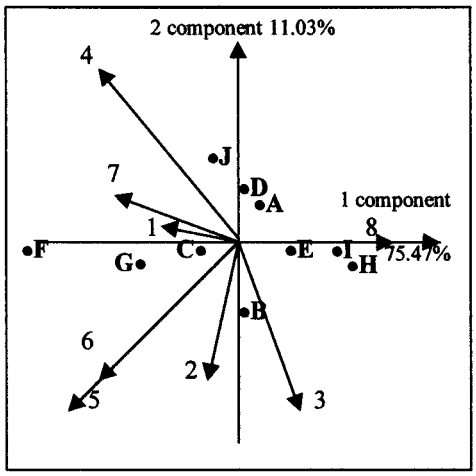

Figure 9. PCA plot of sensory profile data of 10 different oil samples. Sample codes: (A) peanut, fresh; (B) soy I, fresh; (C) soy II, stored; (D) rapeseed/soy, stored; (E) rapeseed refined, fresh; (F) rapeseed cold-pressed, stored; (G) linseed, stored; (H) sunflower, fresh; (I) corn, fresh; (J) olive oil, stored. Descriptors: 1) acidic; (2) metallic; (3) sweet; (4) green; (5) oxidized; (6) musty; (7) earthy; (8) desirability.

Similarly to the samples analyzed in accelerated storage experiment, all 10 samples of different oils were coded and analyzed by sensory panel. Data obtained after statistical interpretation by PCA have been presented in Figure 9. The sensory profile analysis showed great differentiation in the odor characteristics of oil samples. Green (4), oxidized (5), and sweet (3) were the most significant odor attributes responsible for the differentiation as well as desirability (8). The most desired samples were E (rapeseed, refined, fresh), I (corn, fresh) and H (sunflower, fresh), which were almost odorless. Extremely undesired sample was sample F (rapeseed, cold-pressed, stored) of strong oxidized (5), musty (6), earthy (7), and acidic (1) odor. The same attributes described samples G (linseed oil, stored) and C (soy II, stored), but they were of lower intensities. The sample B (soy I, fresh) characterized mostly metallic (2), sweet (3), and little oxidized (5) odor, while samples J (olive oil, stored), D (rapeseed/soy, stored), and A (peanut, fresh) were of similar medium sensory quality—mostly green (4), sweet (3), and earthy (7).

DISCUSSION

Solid-phase microextraction has found applications in a variety of fields—the environmental analysis and flavor analysis being the major ones. The number of publications in the field of food chemistry roughly doubles every 2 years. However, information on applications of SPME in determination of flavor compounds originating from lipids decomposition are very limited. Keszler et al. (1998) used PDMS fiber in their investigation of sunflower oil volatiles and identified 13 compounds using ion trap mass spectrometer after extraction at 40 °C, and in oil samples exposed to air analyzed after 5 weeks of storage, they detected as main products hexanoic and decanoic acids.

The crucial step in the development of SPME method is the proper choice of fiber coating. Fibers coated with phases containing Carboxen seem to perform best in analyses where sensitivity is an issue. The chosen CAR/PDMS/DVB coating offered both low detection limits and satisfactory linearity even in cases when compounds were quantified in a broad concentration range. Limits of detection in SPME headspace analysis can be usually lowered by increasing extraction temperature, thus easing off the migration of volatiles into headspace phase from liquid matrix. Plant oils undergoing autoxidation are especially sensitive to heating, as both intermediates in autoxidation reactions (hydroperoxides) and their decomposition products are labile and prone to changes. In all methods where volatile compounds are determined, sample heating has been involved (Ulberth and Roubicek, 1995; Medina et al., 1999) and samples are heated sometimes to temperatures exceeding 100 °C (Warner and Nelsen, 1996). Snyder (1995) proposed supercritical carbon dioxide to extract volatiles from canola, corn, soybean, and sunflower oils at 50 °C, as a way to decrease the changes in the sample preparation step related to elevated temperatures.

By performing SPME extraction at 20 °C, undesired reactions are slowed or eliminated. Therefore, this temperature is recommended when volatile compounds in fresh oils are to be measured. In measurement of volatile compounds emitted in accelerated storage test, sampling temperature is not that important as the compounds eventually generated during sampling are only a small percentage of all volatiles.

Due to affinity of volatiles to oil matrix and the character of compounds, the time required to reach equilibrium in a vial can be relatively long, especially at 20 °C.

SPME extraction at 50 °C and compound identification by GC−MS revealed 56 main components present, usually both in refined and cold-pressed rapeseed oils. Octanal and nonanal are the main volatile carbonyls derived from oleic acid, the most abundant in rapeseed. Hexanal is the main product of linoleic acid oxidation. Other most abundant aldehydes formed in this process are (*E*)-2-heptenal, (*E*)-2-octenal, and 2,4-decadienals. From oxidation of linolenic acids, the following main carbonyl compounds originate: 2,4-heptadienals, 2-pentenal, and (*Z*)-3-hexenal—the last one not identified in our study (Frankel, 1985; Grosch 1982). Hexanal, 2-heptenal, 2,4-heptadienal, 2-octenal, nonanal, 2,4-nonadienal, and decadienal were detected in rapeseed oil by Guth and Grosch (1990) when authors investigated odoriferous compounds of this oil stored in daylight at room temperature using aroma extract dilution analysis. In our study, only one odorant, (*E*)-2-nonenal, of relatively high FD value (FD = 32) described by Guth and Grosch was identified in rapeseed oil subjected to accelerated storage test. Aldehydes forming the majority of volatiles in samples analyzed by GC−MS are responsible for the oily odor of samples. For oxidized taint, 2-heptenal, 2,4-heptadienal, 1-octene-3-one, and 1-octanol are responsible, and the first two of them were detected in stored oils in our test. (*E,Z*)-2,4-Decadienal is responsible for sweet note in oil odor and 2-hexenal and (*E*)-4-heptenal for the green/grassy notes (Saxby, 1982).

Snyder (1995) identified and quantified volatile compounds in accelerated storage tests using different oils and supercritical fluid extraction for compounds isolation. After 16 days of storage at 60 °C, prevailing compounds in rapeseed oil were hexanal (52.6 ppm), nonanal (27.0 ppm), octanal (20.4 ppm), and decadienal (17.0 ppm). The amounts correspond with aldehydes quantified in our study (Figure 6) with the exception of 2-heptenal, which was predominant aldehyde in 10 day

samples in our study. Total amount of volatiles increased during storage test, regardless of the temperature of sampling. This occurred for both cold-pressed and refined rapeseed oils.

When amount of volatiles was compared with sensory evaluation, it was observed that samples where the amount of volatiles was the lowest were perceived as the most desired. When monitored aldehydes were considered, the rapid increase in their contents paralleled the decrease in desirability of samples. The main descriptors for differentiated stored samples were oxidized (which can be influenced by 2-heptenal contents), acidic (presence of free fatty acids), and green (caused among other compounds by 2-hexenal). The high contents of aldehydes, especially 2-heptenal, correlated with the qualification of refined rapeseed oil stored for 10 days as the worst sample. On the other hand samples with low content of aldehydes and also total volatiles (fresh refined, fresh cold-pressed and 3 day stored cold-pressed rapeseed oils) were evaluated as best, though the cold-pressed oils were characterized by a distinct sweet note. This note can be related to 2,4-decadienal or hexanal presence (Saxby, 1982; Angerosa et al., 2000).

Sensory analysis results for various oils also indicated the relation between volatile compounds contents and oil desirability. Though only a group of 14 compounds was considered, the high amount of volatiles indicated the low acceptance of sample, where oils almost odorless were perceived as the best ones. From both the accelerated storage test and comparison of various oils, it can be concluded that the desirability of oil is associated with amount of volatile compounds present in it. The relation was observed when total volatiles, or selected compounds—most representative of them were aldehydes—were monitored. As observed, the amount of volatiles can vary in a broad range, depending on oil type, its freshness, and condition of storage. The amount of detected constituents after storage in darkness at ambient temperature can be in a range of low micrograms per liter, but can also reach concentration of high milligrams per liter when oil subjected to accelerated stability tests is analyzed.

Analysis of volatile compounds is often ranked second after sensory analysis to evaluate the lipid oxidation in terms of their usefulness (Frankel, 1993). As a result, numerous methods used for compounds isolation exist, based mostly on a headspace analysis (Przybylski, Eskin, 1992). Developed new SPME method allows simple and rapid determination of odoriferous compounds present in oils at very low concentrations at ambient temperature. Contrary to static or dynamic headspace methods it does not require costly equipment.

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