Determination of volatile organic compounds in the crude and heat treated amaranth samples

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ABSTRACT: The present study concentrated on the development of an analytical method for determination of emissions of volatile organic compounds from crude and heat treated amaranth (genus *Amaranthus* L.) samples. Emitted substances were collected by solid-phase microextraction (SPME) method and identified by gas chromatography with mass spectrometry. The list of identified abundant organic compounds exceeds one hundred substances of different classes. Total concentrations of quantified volatile organic compounds ranged between 2.2 and 68.9 μg/g of dried sample. Hexanal and acetic acid were found as the most abundant compounds detected in amaranth samples. It was found that heat treatment (popping) of amaranth samples changed their composition of volatile organic compounds dramatically. The highest volatile organic compound emissions were found in popped grain amaranth in comparison to all crude grains and amaranth biomasses.

Keywords: amaranth; volatile organic compounds (VOCs); heat treatment; GC/MS; HS-SPME

Species of the genus *Amaranthus* (L) are herbaceous plants distributed throughout the world. Both the seed and vegetative growth have been used for food. Nutritional evaluation of grain amaranth and forage indicate a high potential for use in animal and human diets (Alfaro et al., 1987; Szelenyi-Galantai and Zsolnai-Harszi, 1992; Andrasofszky et al., 1998; Pisarikova et al., 2006). The dry matter of grain amaranth contains 12.6 to 18.0% of proteins, 5 to 8% of fat, 60 to 65% of saccharides, and 3 to 5% of crude fibre (Yanez et al., 1994). Grain amaranth is rich in lysine and sulphur amino acids. Amaranth oil is rich in unsaturated fatty acids, especially linoleic and oleic acids; the content of squalene (5 to 6%) is also important. The nutritional value of the above-ground biomass depends on the growth stage of plants. The contents of crude protein ranged from 16.3 to 29.5%, crude fiber from 11.1 to 24.4%, fat from 2.0 to 3.0% and ash from 13.1 to 17.8% in dry matter (Alfaro et al., 1987; Zeman et al., 1995).

The use of amaranth for diets is partly limited by presence of antinutritional substances – trypsin inhibitor, phenols, tannins, and fytohaemagglutinins (Correa et.al., 1986; Imeri et al., 1987). It was shown that antinutritional substances could be partially or totally degraded by heat treatment, i.e. autoclaving, popping and extruding (Andrasofszky et al., 1998). Heat treatment resulted in an increase in fiber content and a decrease in nitrogen-free extracts (Hoover and Vasathan, 1994). Heat-treated grain amaranth contained more proteins, fat, ash and gross energy. Heat treatment induces the inactivation of antinutritional factors but on the other hand it may cause amino acid degradation, formation of intramolecular bonds and Maillard reactions, which impair digestibility of nutrients (Nestares et al., 1993; Pisarikova et al., 2005).

All volatile organic compounds (VOCs) emitted from plants can originate from biogenic and/or anthropogenic sources. Many plants emit substantial amounts of phytogenic volatile organic compounds

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(PVOCs), which include alkanes, alkenes, alcohols, aldehydes, ethers, esters and carboxylic acids. Defense, communication and/or protection against extreme environmental conditions have been proposed as reasons for these emissions. PVOCs are produced by a range of physiological processes in many different plant tissues and are themselves also extremely diverse, more than 30 000 compounds were predicted (Niinemets et al., 2004). At a global scale, the emission of plant volatiles exceeds by several-fold these emissions from man-made sources (Penuelas and Llusia, 2004). Besides that, plant foliage is a natural passive sampling medium for collection of atmospheric dry or wet deposition of pollutants. There is a little information about toxicity of VOCs emitted in agriculture. Therefore, chemical identification and quantification is the necessary step in risk assessment of these compounds.

Over the past years, headspace solid-phase microextraction (HS-SPME) has been widely employed for the analysis of volatile organic compounds (VOCs) in a variety of complex matrixes such as environment, food, and biomedical solid samples (Jia et al., 1998; Mills and Walker, 2000; Ulrich, 2000; Zygmunt et al., 2001; Bicchi et al., 2004; Li et al., 2005; Carrillo et al., 2006).

HS-SPME is a solvent-free sample preparation technique in which a fused silica fiber coated with polymeric organic liquid is introduced into the headspace above the sample. The volatilized organic analytes are extracted and concentrated in the coating and then transferred to the analytical instrument for desorption and analysis. This modification of the solid-phase microextraction method (SPME) shortens the time of extraction and facilitates the application of this method to analysis of solid samples (Zhang and Pawliszyn, 1993). SPME sampling device can operate in the linear (kinetic) or equilibrium uptake regimes (Wania et al., 2003).

Development of analytical method and its optimization for VOCs determination in the plant samples was the aim of this study. This method was used for grain amaranth and dried biomass analyses of VOCs emissions before and after heat treatment.

MATERIAL AND METHODS

Chemicals

EPA 524.2 VOCs Mix standard was supplied by SUPELCO (Bellefonte, PA, USA). The other chemicals used were of the highest purity available. SPME fibers:

carboxen/polydimethylsiloxane, film thickness 70 μm; polydimethylsiloxane/divinylbenzene, film thickness 65 μm and divinylbenzene/carboxen/polydimethylsiloxane, film thickness 50/30 μm were from SUPELCO (Bellefonte, PA, USA).

Sample collection, preparation and extraction

Amaranth used in the experiments of the present study was provided by the company AMR Amaranth, Ltd. (Hradec Kralove, Czech Republic). Grain of three amaranth varieties (*Amaranthus cruentus* – K 283, Olpir and *A. hypochondriacus* – K 432) was analyzed prior to and following heat treatment, and the above-ground biomass of five varieties (*Amaranthus cruentus* – K 283, Olpir, *A. caudatus* – Elbrus, *A. hypochondriacus* – K 432, No. 1008) was analyzed in the stage of milk (waxy ripeness). The plants were harvested in September 2006. The harvest was stored at the requested humidity of 12%. Crude grain amaranth (AC) was directly sampled to cleaned head-space 60 ml vials. Heat treated (popped) grain amaranth (AP) was heated at 170°C for 30 second. Heat treatment of amaranth grain (popping, extrusion, puffing, cooking etc.) is used to reduce the content of antinutritional substances and diminish their effects. The most common way of grain treatment is "roasting" (popping) at 160°C to 170°C under usual or increased pressure. The grain cracks, increases its volume and obtains nutty flavor. The treated grain is then used as a supplement of rational nutrition. However, heat treatment can result in destruction of protein and lipid component of amaranth and formation of undesirable substances due to the Maillard reaction. Dried biomass (AB) was obtained by drying and subsequent grinding of the above-ground biomass at the milky ripe stage. Both types of samples were immediately collected to cleaned vials. Eleven samples of amaranth were used for VOCs analysis. Five of them were dried biomass (designated AB1–AB5), three were crude grain amaranth (AC1, AC2, AC3) and the same samples were heat treated grain amaranth (AP1, AP2, AP3).

Extraction of samples using HS-SPME was accomplished by placing the fiber in the headspace above the sample in 60 ml headspace glass vials.

The following steps were performed so as to properly identify and quantify VOCs in the amaranth samples using HS-SPME:
– determination of conditions for analysis of VOCs by a selected analytical technique (GC/MS)
– selection of appropriate SPME fiber
– determination of optimal extraction time and temperature
– testing reproducibility of SPME analysis

GC/MS analysis

VOCs analysis using GC/MS was based on a combination of determination of retention times and relative abundances of selected ions. GC separation was performed in a CP-Select 624 CB fused silica capillary column (60 m, 0.25 mm I.D., d_f = 1.4 μm; Varian, Walnut Creek, CA, USA). Helium in a column head at pressure of 70 kPa was used as the carrier gas. An ion trap mass spectrometer Saturn 2100T (Varian, Walnut Creek, CA, USA) was used for the detection and identification of the analytes. The mass spectrometer was operated in an electron ionization (EI) mode at electron ionization energy of 70 eV.

Quality assurance

Washed glassware was rinsed with acetone and hexane and, in some cases, heated in a muffle furnace (450°C, 4 h) to remove any traces of volatile organic compounds. Quality assurance samples included spiked matrixes, spiked controls, procedure blanks and calibration standards in methanol.

Phytogenic volatile organic compounds released by amaranth samples were termed as PVOCs and other emitted substances as other volatile organic compounds (oVOCs). Then VOCs (volatile organic compounds) mean the sum of respective PVOCs and oVOCs abundances or concentrations.

GC/MS identification was done by comparison of retention times and searching with mass spectra databases of these compounds. Total emissions of VOCs (termed Abundance in the figures) were expressed as a sum of abundances of all identified abundant organic compounds converted to 1 g of the dried sample. The method of standard addition of known standard amount directly to the measured matrixes immediately before HS-SPME analysis was used for quantification of selected compounds.

RESULTS AND DISCUSSION

Determination of optimal analytical conditions

The linear or equilibrium regimes of SPME accumulation of organic compounds are demonstrated on benzene emitted from amaranth sample (Figure 1). From practical point of view, the linear regime was used for HS-SPME determination of VOCs in the samples. Therefore the used sampling time was 20 min. For SPME that operate in the linear regime, the rate of mass transfer to the polymer phase is linearly proportional to the difference between the chemical activity of the analyte in the head-space and the used SPME phase. Results summarized in Figure 2 show that carboxen/polydimethylsiloxane coated fiber extracts (except for hexanoic acid) selected compounds much better than other tested SPME fibers. Extracted amount of benzene increased with increasing extraction temperature (Figure 3). According to the experimental conditions and used laboratory equipments, 50°C was chosen as extraction temperature. Reproducibility expressed as relative standard deviation (RSD) calculated from three parallel determinations of selected VOCs by optimized HS-SPME analytical method ranged from 5.4% (*o*-xylene) to 20.7% (naphthalene). According to the above mentioned information, analytical method with the following conditions was developed. This method is useful for determination of changes originating from amaranth heat treatment and can be used for characterization of VOCs composition in different types of amaranth samples.

Optimal conditions for the analytical method:
– Samples: amaranth without further laboratory modifications in 60 mL HS glass vials

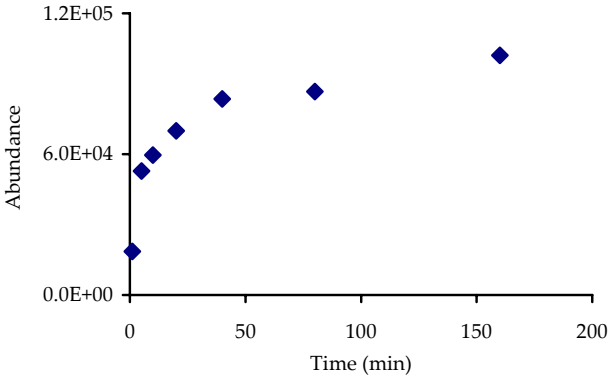
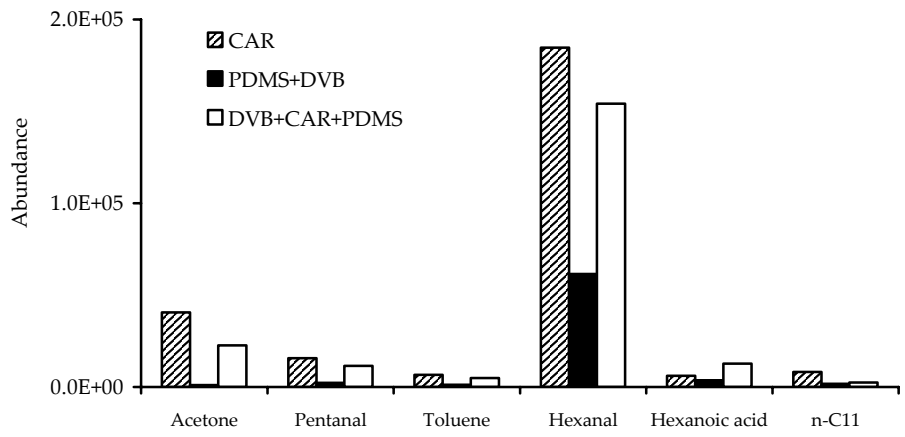


Figure 1. Kinetics of HS-SPME extraction of benzene from amaranth sample (AC3) at 50°C



CAR = carboxen/polydimethylsiloxane, 70 µm;
PDMS + DVB = polydimethylsiloxane/divinylbenzene, 65 µm;
DVB + CAR + PDMS = divinylbenzene/carboxen/polydimethylsiloxane, 50/30 µm

Figure 2. Extraction efficiency of three types of SPME fibers for HS-SPME extraction of selected compounds from amaranth sample (AC3) at 50°C and extraction time 20 min

– SPME fiber: carboxen/polydimethylsiloxane, film thickness 70 µm
– Extraction: headspace, 50°C, 10 min temperature equilibration, 20 min extraction
– Desorption time and temperature: 10 min, 260°C
– GC column: CP-Select 624 CB (60 m, 0.25 mm I.D., d_f = 1.4 µm)
– GC carrier gas: helium, 1.0 ml/min, constant flow rate mode
– GC oven temperature program: 35°C (2 min); 10°C/min 200°C; 20°C/min 250°C (10 min)
– GC injector temperature: 260°C
– GC injection mode: splitless (2 min)
– GC injector liner: type for SPME, 0.75 mm I.D.
– MS interface temperature: 260°C
– MS mode: scan, mass range 35–260 m/z

Figure 4 shows a typical GC/MS chromatogram of volatile organic compounds released from amaranth sample (AP3) measured by optimized HS-SPME method.

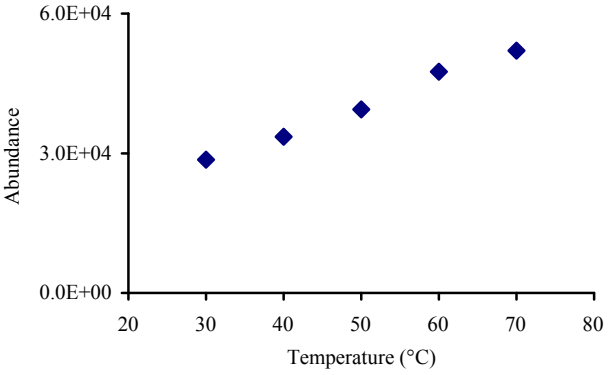


Figure 3. Influence of extraction temperature on the amount of HS-SPME extracted benzene from amaranth sample (AC3) at extraction time 20 min

Occurrence of volatile organic compounds in amaranth samples

More abundant volatile organic compounds emitted from the amaranth samples are listed in Table 1. According to this list, the oxygenated compounds were emitted from amaranth samples mainly by physiological processes (PVOCs) contrary to the aromatic compounds emitted mainly from anthropogenic sources (oVOCs). It was found (see Figure 5) that amaranth biomass (AB1 – AB5) emitted more VOCs than crude grain amaranth (AC1 – AC3). In all samples, the predominating emitted compounds were PVOCs. The relative contents of these compounds ranged from 62.5% (AC2) to 82.2% (AC1) in crude grain amaranth and from 86.5% (AB1) to 93.6% (AB3) in amaranth biomass. In the case of crude grain amaranth, no significant differences ($P > 0.1$) between respective samples were found in the emission of selected VOCs. On the contrary, significant differences ($P < 0.05$) were found between emissions from crude grain and biomass amaranth samples.

The analytical method HS-SPME revealed differences in emissions of the selected classes of VOCs from crude grain and amaranth biomass samples. These results are demonstrated in Figure 6 that shows differences in relative content of volatile aromatic compounds (BTEX), alkylbenzenes, alkanes, alcohols, ketones, aldehydes and carboxylic acids.

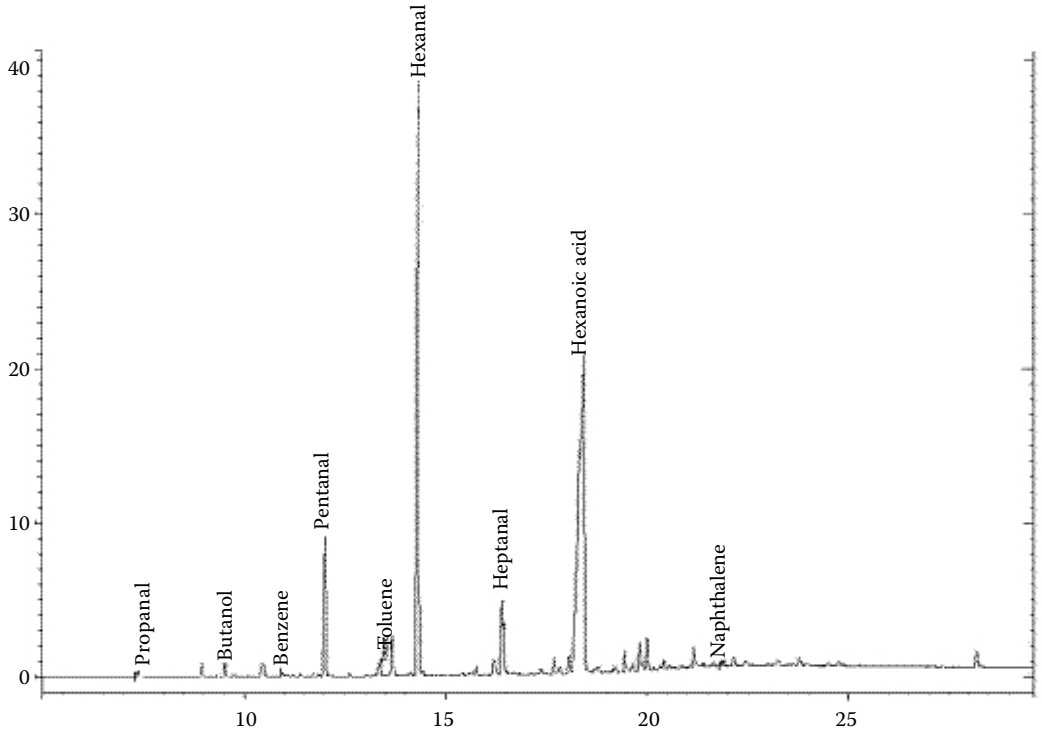


Figure 4. Typical GC/MS chromatogram of HS-SPME analysis of oVOCs in selected amaranth sample (AP3)

The highest relative quantity of aldehydes (48.3%, AC1) was emitted from crude grain and carboxylic acids (52.8%, AB2) from amaranth biomass samples.

Influence of heat treatment on the VOCs emission

Heat treatment (popping) of crude grain has been frequently used as a modification of amaranth feedstuff preparation. Figure 7 shows what arouse during this procedure, when crude grain amaranth (AC1 – AC3) was heat treated with the aim to produce the popped grain (AP1 – AP3). Crude grain opened due to this process and VOCs could release from them easier than before this treatment. VOCs emission increased 7.8 times for AC1 and AP1, 29.6 times for AC2 and AP2 and 41 times for AC3 and AP3 samples. Except for AC1 and AP1, heat treatment increased relative amount of BVOCs from 62.5% (AC2) to 85.2% (AP2) and from 63.1% (AC3) to 93.8% (AP3).

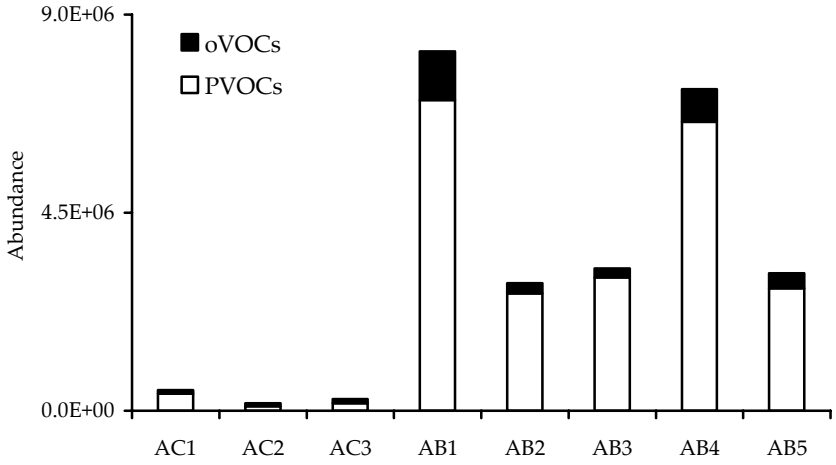


Figure 5. Total emission of PVOCs and oVOCs from amaranth crude grain (AC) and biomass (AB) samples

Table 1. List of more abundant volatile organic compounds emitted from amaranth samples

O-compounds	
Alcohols	isopropyl alcohol; 4-penten-2-ol; 2-methyl-3-buten-2-ol; 5-nonanol; 2-furanmethanol; ethyl acetate; methylbutyloxirane
Furans	furan; 2,5-dihydrofuran; methylfuran; 2-pentylfuran; 2-methylfuran; 2-ethylfuran; 2-*n*-butylfuran
Phenols	phenol; 4-methylphenol; 2-ethylphenol
Ketones	acetone; -pentanone; 2-heptanone; furanylethanone; 2-methylheptenone; 1-methylheptenone
Aldehydes	propanal; 2-methylpropanal; butanal; 2-methylbutanal; 3-methylbutanal; pentanal; hexanal; heptanal
Carboxylic acids	acetic acid; propanoic acid; butanoic acid; methylbutanoic acid; entanoic acid; meethylhexanoic acid; hexanoic acid; heptanoic acid
N-compounds	methylpyrazine; 2,5-dimethylpyrazine; 2,3-dimethylpyrazine
S-compounds	dimethyldisulfide
Aromatic hydrocarbons	
BTEX	benzene; toluene; ethylbenzene; *m*+*p*-xylene; *o*-xylene; styrene
Alkylbenzenes	isopropylbenzene; *n*-propylbenzene; 1-ethyl-3-methylbenzene; 1-ethyl-4-methylbenzene; 1,3,5-trimethylbenzene; 1-ethyl-2-methylbenzene; 1,2,4-trimethylbenzene; *t*-butylbenzene; *sec*-butylbenzene; *iso*-butylbenzene; *p*-isopropyl-toluen; 1,2,3-trimethylbenzene; 1,3-diethylbenzene; *n*-butylbenzene; 1-meethyl-3-*n*-propylbenzene; 1,4-diethylbenzene; 1,4-dimethyl-2-ethylbenzene; 1,3-dimethyl-4-ethylbenzene; 1,2-dimethyl-4-ethylbenzene; 2,3-dimethyl-1-ethylbenzene; 1,2,4,5-tetramethylbenzene; 1,2,3,5-tetramethylbenzene; 1,2,3,4-tetramethylbenzene
PAHs	indene; DiH-1H-indene; 1,2,3,4-tetraH-naphthalene; naphthalene; 2-methylnaphthalene; 1-methylnaphthalene
Halogenated compounds	methylene chloride; chloroform; carbon tetrachloride; trichloroethylene; tetrachloroethylene
Alkanes	2-methylpentane; hexane; methylhexane; 2-methyl-1-propene; methylcyclohexane; *n*-heptane; dimethylcyclohexane; 4,5-dimethyl-1-hexene; ethylmethylcyclohexane; *n*-undecane; *n*-dodecane; *n*-tridecane; *n*-tetradecane; *n*-pentadecane

Figure 8 shows differences in VOCs emissions from the same amaranth species in the form of crude grain (AC3), popped grain (AP3) and dried biomass (AB5). The highest emission was found for popped grain (AP3); it was 41 times higher than for crude grain (AC3) and 3.4 times higher than for biomass. Relative contents of PVOCs were 63.1% for crude grain (AC3), 93.8% for popped grain (AP3) and 88.9% for biomass sample (AB5).

Figure 9 shows differences in relative contents of selected classes of volatile organic compounds in the same amaranth species. Carboxylic acids were emitted predominantly (62.1%) by popped grain (AP3) and amaranth biomass (48.9%, AB5). Emission of aldehydes by crude amaranth samples prevailed (33%, AC3). Aldehydes were also the second most emitted class of volatile compounds by the popped grain and amaranth biomass.

It can be seen from the present results that the levels of VOCs emissions from grain amaranth and biomass are temperature dependent. The lipid compound of grain amaranth is rich in unsaturated fatty acids, among which linoleic acid represents 50% (Lorenz and Hwang, 1985). Linoleic acid is a precursor of saturated and unsaturated aldehydes and a number of other compounds (Nawar, 1996).

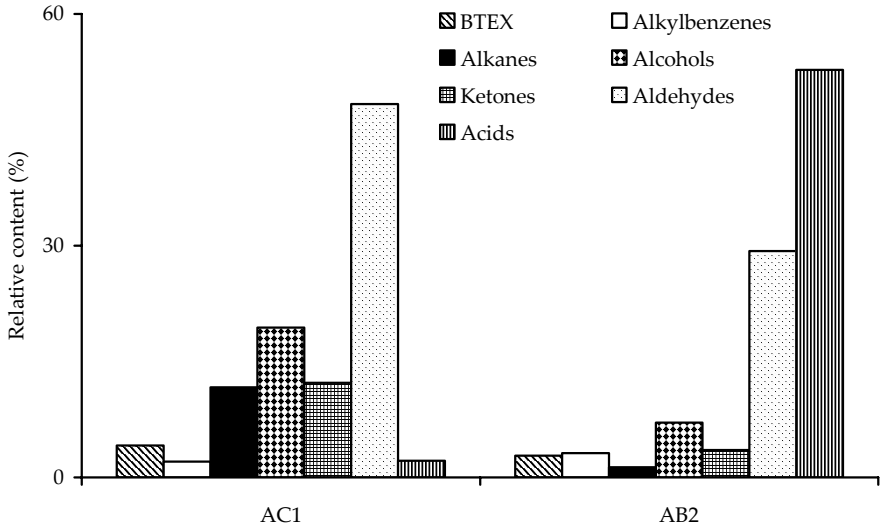


Figure 6. Differences in relative content (%) of selected classes of organic compounds between crude grain (AC1) and biomass (AB2) amaranth samples

Concentrations of selected volatile organic compounds (including fatty acids and aldehydes) in the amaranth samples are shown in Table 2. Concentrations of hexanal and acetic acid were the highest among all the quantified substances. Total concentrations of quantified compounds ranged between 2.2 and 68.9 μg/g.

Hexanal is viewed as indicator of oxidative state in a number of foodstuffs (Sanches-Silva et al., 2004). High contents of hexanal after heat treat-

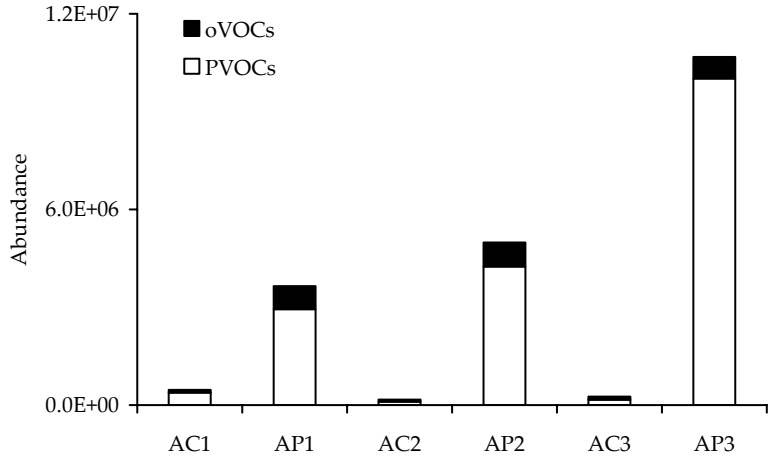


Figure 7. Influence of heat treatment on PVOCs and oVOCs emission from grain amaranth samples (AC1, AC2, AC3 – crude grain; AP1, AP2, AP3 – popped grain samples)

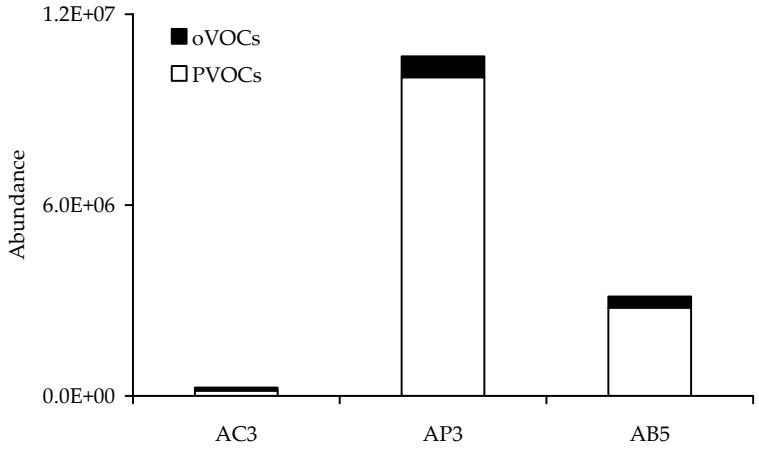


Figure 8. Total emission of volatile organic compounds from the same amaranth species (crude grain – AC3, popped grain – AP3 and granulated biomass – AB5)

Table 2. Concentration of selected compounds in the amaranth samples (ng/g)

Compound name	Sample name										
	AC1	AP1	AC2	AP2	AC3	AP3	AB1	AB2	AB3	AB4	AB5
Methylene chloride	6.28	42.3	9.04	33.6	3.85	9.68	7.61	5.34	1.34	59.6	5.83
Chloroform	55.2	854	75.4	478	144	60.1	263	228	23.4	356	79.5
Benzene	13.1	55.2	7.70	62.6	34.3	186	66.9	24.4	15.4	108	165
Trichloroethylene	7.60	36.3	5.90	15.0	66.0	321	488	8.73	4.12	69.6	294
Toluene	58.6	695	97.0	648	251	798	1 165	307	592	764	631
Hexanal	2 483	16 674	462	23 414	822	23 851	22 861	8 555	14 203	19 399	10 473
Ethylbenzene	15.6	126	18.3	65.8	20.8	67.7	237	76.1	47.0	180	77.8
m+*p*-Xylene	27.9	217	28.8	107.5	34.4	128	469	129	64.1	308	136
o-Xylene	16.6	232	29.9	104.3	36.0	126	469	137	81.1	388	137
Styrene	42.5	32.2	18.2	21.6	9.67	30.1	358	58.4	75.8	147	81.5
Naphthalene	986	1 183	894	2 114	689	2 359	6 802	788	1 263	3 832	2 923
Acetone	282	1 115	106	1 987	101	1 688	2 917	187	452	582	323
Acetic acid	14.2	1 611	216	3 184	61.1	3 109	7 391	15 148	8 389	34 753	13 811
3-Methylbutanal	18.3	483	3.80	513	11.8	41.9	2 068	163	22.8	217	61.9
2-Methylbutanal	12.1	608	3.98	1 053	10.3	57.3	1 246	363	584	387	167
2-Methyl-1-propene	27.9	192	15.6	151	34.7	193	201	34.0	14.5	278	39.1
2-Pentanone	13.6	31.6	3.82	64.0	2.97	91.3	803	29.0	40.4	36.0	23.6
5-Nonanol	138	1 390	26.6	2 215	54.3	4 665	8 139	1 028	1 695	1 447	888
Pentanal	109	729	29.3	998	52.8	1 594	2 816	413	647	841	412
Propanoic acid	0.20	5.71	0.71	24.1	1.23	133	1 665	193	370	926	252
4.5-Dimethyl-1-hexene	152	1 940	22.9	2 226	62.7	1 976	489	84.8	213	510	327
2-Heptanone	52.0	871	27.6	745	86.2	1 006	689	138	518	434	266
Heptanal	9.80	87.9	5.93	87.3	10.6	515	286	93.2	211	374	161
Pentanoic acid	4.75	37.4	5.40	45.7	6.12	325	231	10.9	53.3	470	62.8
2.5-Dimethypyrazine	4.94	3 140	7.93	4 234	12.1	215	91.14	21.7	46.7	398	49.7
Hexanoic acid	65.1	474	30.6	554	143	549	1 141	257	68.4	302	191
Me-Heptenone	19.0	358	3.87	622	9.26	366	1 503	404	2 355	1 318	663
Sum of concentrations	4 635	33 221	2 156	45 768	2 771	44 460	64 864	28 885	32 050	68 885	32 700

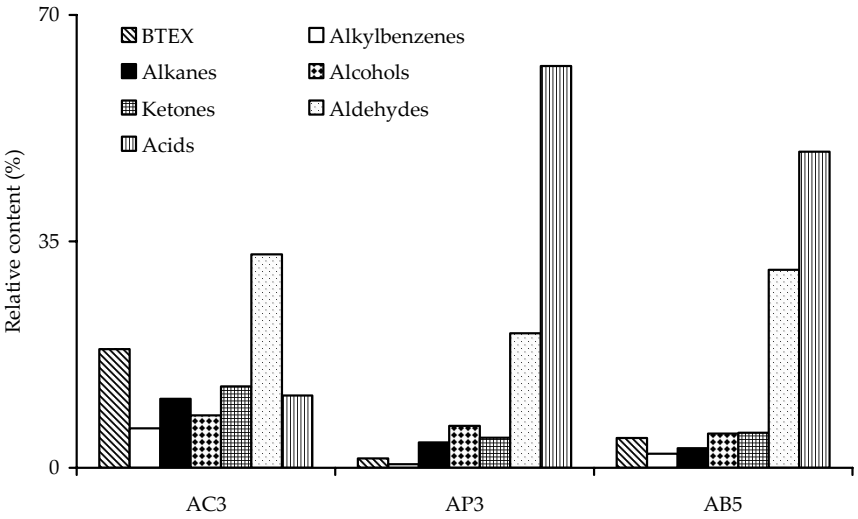


Figure 9. Differences in related content (%) of selected classes of organic compounds in the same amaranth species

ment was detected in spice paprika (Cremer and Eichner, 2000), leek (Nielsen et al., 2004) and extracted poppy oil (Krist et al., 2005). Hexanal odour activated hypothalamic nuclei, which control maternal and emotional behaviour (Hamaguchi-Hamada et al., 2004). Additional data concerning the effect of VOCs on physiology of digestion is missing at present.

CONCLUSIONS

The HS-SPME GC/MS method facilitated determination of volatile organic compound emissions from crude and popped grain amaranth and its biomass. This up to date analytical method is very effective and accuracy procedure for analysis of mentioned matrixes.

In this study, significantly increased VOCs emissions from popped (heat treated) grain amaranth were found. These VOCs emissions were higher than emissions from other crude grain and amaranth biomasses. PVOCs constituted the main part (> 80%) of all analyzed volatile organic compounds.

Hexanal and acetic acid were found as the most abundant substances analyzed in all amaranth samples under study, however toxicity characterization of VOCs and heath risk assessment should be performed.

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