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Analytical Methods

Aroma profiles of five basil (*Ocimum basilicum* L.) cultivars grown under conventional and organic conditions

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Abstract

A headspace solid-phase microextraction (HS-SPME) method coupled to gas chromatography–ion trap mass spectrometry (GC–ITMS) has been developed and applied for profiling of volatile compounds released from five *Ocimum basilicum* L. cultivars grown under both organic and conventional conditions. Comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC–TOFMS) was employed for confirmation of identity of volatiles extracted from the basil headspace by SPME.

Linalool, methyl chavicol, eugenol, bergamotene, and methyl cinnamate were the dominant volatile components, the relative content of which was found to enable differentiating between the cultivars examined. The relative content of some sesquiterpenes, hydrocarbons benzenoid compounds, and monoterpene hydrocarbons was lower in dried and frozen leaves as compared to fresh basil leaves.

A sensory analysis of the all examined samples proved the differences between evaluated cultivars.

Keywords: Solid-phase microextraction (SPME); Basil; *Ocimum basilicum* L.; Ecological farming; Volatiles; Terpenes; Gas chromatography (GC); Ion trap mass spectrometry (ITMS); Comprehensive two-dimensional gas chromatography (GC×GC); Time-of-flight mass spectrometry (TOFMS)

1. Introduction

Ocimum basilicum L. (basil) belonging to the plant family *Lamiaceae*, subfamily *Nepetoideae*, and the genus *sensu lato*, comprises 65 species (Paton, Harley, & Harley, 1999). Basil is an annual, 20–60 cm long, white-purple flowering plant, which is originally native to India and other regions of Asia. Basil leaves containing essential oils of distinctive aroma can be used both fresh and dried to spice up various kinds of meals. Apart of culinary use, basil has been traditionally employed as a medicinal herb in the treatment of headaches, coughs, diarrhoea, constipation, warts, and/or kidney malfunction (Grayer et al., 2004; Özcan, Arslan, & Ünver, 2005; Politeo, Jukica, & Milosa, 2007).

The presence of essential oils and their composition determine the specific aroma of plants and the flavour of the condiment. Not only the type of cultivar but also the agronomical practices and environmental conditions affect the composition of sensory important compounds (Jirovetz, Buchbauer, Shafi, & Kaniampady, 2003; Viña & Murillo, 2003). Regardless of these factors, 1,8-cineole, methyl cinnamate, methyl chavicol, and linalool (Lee, Umano, Shibamoto, & Lee, 2005) are generally the main compounds responsible for the typical basil aroma. On the basis of more than 200 analyses of essential oils isolated from *O. basilicum* L., Lawrence (1988) classified four major essential oil chemotypes of basil: (1) methyl chavicol-rich, (2) linalool-rich, (3) methyleugenol-rich, (4) methyl cinnamate-rich, and also numerous subtypes.

Several analytical methods have been developed to determine volatile constituents of essential oils present in spices.

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Representing a complex mixture of volatile substances (typically monoterpenes, sesquiterpenes, and their oxygenated derivatives) present generally at low concentration in plants (Diaz-Maroto, Peréz-Coello, & Cabezudo, 2002; Lucchesi, Chemat, & Smadja, 2004), the essential oils have to be extracted and concentrated within the analytical procedure. Various methods, such as steam distillation, solvent extraction (often employing Soxhlet apparatus) or simultaneous distillation-extraction (SDE), and head-space methods, solid-phase microextraction (SPME) included, have been used to analyse volatile compounds in plants or spices. Nevertheless, it should be noted that the use of "classic" extraction/distillation methods can cause some difficulties: monoterpenes are prone to undergo chemical changes under conditions of a steam distillation, and, a conventional solvent extraction is likely to involve losses of the most volatile compounds during the step of solvent removal (Lucchesi et al., 2004; Luque de Castro, Jiménez-Carmona, & Fernández-Pérez, 1999; Pollien, Ott, Fay, Maignial, & Chaintreau, 1998). Ideally, the methods used for flavour analyses should not only avoid changes in natural flavour pattern, but, should be also fast, if possible solvent-less, amenable to automation, and inexpensive.

These criteria are met by SPME, introduced and later developed by Pawliszyn and co-workers (Artur & Pawliszyn, 1990; Pawliszyn, 1997). This technique is highly appreciated by the food industry for the analysis of volatile compounds (Jirovetz, Buchbauer, Ngassoum, & Geissler, 2002; Odeh et al., 2007; Romeo, Ziino, Giuffrida, Condurso, & Verzera, 2007) due to the rapid sample analysis both in the laboratory or on-site where the investigated system is located. The SPME procedure combines extraction and concentration of analytes in one step using a simple device. The plant volatiles are concentrated onto a fibre coated with appropriate stationary phase and subsequently desorbed into the hot GC injection port for following gas chromatographic analysis. An equilibrium between the amount of analyte in the sample matrix, in the headspace above the sample, and in the stationary phase coated on a fused silica fibre is established in the case of HS-SPME. The quantity of a given compound adsorbed/absorbed depends both on the partitioning of the compound between the headspace and the plant matrix and the partitioning between the fibre coating and the headspace. SPME technique, which reduces the chemical changes of analytes and artefact formation, combined with gas chromatography–mass spectrometry (GC–MS), has been recognised as a powerful, solvent-free method, suitable for analysis of volatile constituents of plant materials.

The aim of this study was to investigate the applicability of HS-SPME for the analysis of basil volatiles and to differentiate between five cultivars of basil grown under organic and conventional conditions on the basis of the information about the volatiles composition. For this purpose, HS-SPME coupled to gas chromatography–ion trap mass spectrometry (GC–ITMS) has been developed and applied for detection and identification of volatile compounds in fresh, frozen, and dried basil. The results were confirmed using a comprehensive two-dimensional gas chromatography employing a time-of-flight mass spectrometric detector (GC×GC–TOFMS). The analyses of volatile components were completed by a sensory evaluation and the determination of essential oil in dried basil samples.

2. Experimental

2.1. Chemicals and materials

The standards of α-humulene (98%), (−)-*trans*-caryophyllene (β-caryophyllene, 99%), β-myrcene (90%), 1,8-cineole (99%), (±)-camphor (95%), and (−)-bornyl acetate (97%) were obtained as neat compounds from Sigma (USA). The standards of (−)-α-pinene (99%), (−)-β-pinene (99%), (+)-3-carene (99%), (−)-camphene (85%), *R*-(+)-limonene (D-limonene, 98%), and (+)-terpinen-4-ol (99%) were purchased as neat compounds from Fluka (USA). The individual stock solutions (2 g L^{-1}) were prepared in toluene (Merck, Germany) and stored at 4 °C. They were used for preparation of standard mixture in toluene (25 mg L^{-1} each).

The SPME fibres coated with (i) polydimethylsiloxane (100 μm, PDMS), polydimethylsiloxane/divinylbenzene (65 μm, PDMS/DVB), (ii) divinylbenzene/Carboxen™/polydimethylsiloxane (50/30 μm StableFlex, DVB/CAR/PDMS), and (iii) Carbowax®/divinylbenzene (65 μm, CW/DVB) were supplied by Sigma–Aldrich (USA). Prior to use, all fibres were conditioned following the manufacturer's recommendations. Each day, before the samples analyses started, a short thermal "cleaning" of the fibres in a GC injector (30 min at 250 °C) and a blank run were performed.

The 10 mL head-space vials (Sigma–Aldrich, USA) were cleaned using sonication (20 min) in water with detergent, followed by 20 min sonication in distilled water and acetone, p.a. (Penta, Czech Republic). Clean vials were after drying in an oven at 220 °C for 4 h stored covered with an aluminium foil.

2.2. Analysed samples

All samples of fresh basil (*O. basilicum* L.) were collected from the open experimental garden governed by the Czech University of Agriculture in Prague (Czech Republic). Plants were harvested during July 2005. Five cultivars of basil: green cultivar I (Prava zelena), green cultivar II (Trpaslici), red cultivar III (Cinamonette), red cultivar IV (Purple Opaal), and red cultivar V (Rot) were analysed. Each cultivar was grown under conditions of organic (Council Regulation (EEC) No. 2092/91) and conventional farming. While no man-made fertilizers or pesticide preparations were used for growing ecological basil, herbicide Synfloran (0.7% w/w) and mineral fertilizer Cerit (8 kg per 100 m^2) were applied in the case of conventional farming.

2.2.1. Samples preparation

2.2.1.1. Fresh basil. After transportation to the laboratory one group of plants was grown on the windowsill and watered each day. Fresh basil leaves were manually plucked and sliced (2 mm slices), the slices then being thoroughly mixed. One gram of representative sample was placed into a 10 mL headspace vial, a magnetic cap was fixed, and the sample was then analysed by HS-SPME–GC–ITMS.

2.2.1.2. Dried basil. The second group of fresh basils was dried immediately after the plants harvesting (laboratory temperature (25 °C), 12 days in a dark air-conditioned room). Dried basil was powdered, 0.5 g of sample was weighted into a 10 mL headspace vial, a magnetic cap was fixed, and the sample was then analysed by HS-SPME–GC–ITMS.

2.2.1.3. Frozen basil. The freshly plucked leaves were stored at −18 °C for 5 months to find out the potential changes of volatiles composition during storage. One gram of frozen leaves was placed into a 10 mL headspace vial, a magnetic cap was fixed, and the sample was then analysed by HS-SPME. The fresh (non frozen) leaves from the same part of plant were analysed using HS-SPME–GC–ITMS (Day 0).

2.3. SPME procedure

In order to develop a SPME procedure for the isolation of basil headspace volatiles, the optimisation of parameters influencing this process such as fibre coating, extraction time and temperature was carried out. Detail comments on conducted experiments are provided in Section 3.

2.3.1. Optimised conditions

Approximately 1 g of fresh, 0.5 g of dried, or 1 g of frozen basil sample was placed into a 10 mL headspace glass vial, and after being capped with a magnetic cap with PTFE/silicon septa, the vial was heated for 10 min at 30 °C. SPME was performed using a StableFlex DVB/CAR/PDMS 50/30 μm fibre for 5 min at 30 °C. The extracted compounds were thermally desorbed at 250 °C for 1 min in the injector port of a gas chromatograph.

2.4. Gas chromatographic analysis

2.4.1. GC–ITMS system

Automated HS-SPME of basil volatiles was performed using a CombiPal multipurpose sampler (CTC Analytics, Switzerland) connected to a GC–ITMS system (Thermo Quest, USA) consisting of a Trace GC 2000 gas chromatograph (Thermo Quest, USA) equipped with a PTV injector (liner volume 95 μL), digital pressure flow control (DPFC) and an ion trap mass spectrometric detector POLARIS Q (Finnigan, USA). This system was used for all SPME experiments except those aimed at confirmation of volatiles identification by means of GC×GC–TOFMS (see Section 2.4.2). An HP-VOC capillary column (60 m length × 0.2 mm I.D. × 1.1 μm film thickness (Agilent, USA)) was employed for the separation of basil volatiles. The initial oven temperature was set at 45 °C for 1 min, then increased to 200 °C at a rate of 15 °C min^{-1}, and finally up to 275 °C at 5 °C min^{-1} and held for 4 min (total GC run time was 30 min). The injector was operated in a splitless mode with 1 min sampling time (splitless period). The injection port, the transfer line, and the ion source temperatures were set at 250 °C, 275 °C, and 200 °C, respectively. The detector was operated in electron ionisation mode (70 eV) and the Segment Scan mode with m/z ranges: 35–70, 71–110, 111–160, 161–220, 221–320, 321–420, 421–520 was applied.

The data were processed using an XCALIBUR (Finnigan, USA) software (v. 1.2.2).

2.4.2. GC×GC–TOFMS system

A Pegasus 4D instrument consisting of an Agilent 6890N gas chromatograph with a split/splitless injector (Agilent, USA), an MPS2 autosampler for automated SPME (Gerstel, Germany), and a Pegasus III high-speed time-of-flight mass spectrometer (Leco Corp., USA) was used for analyses of some samples. Inside the GC oven a cryogenic modulator and the secondary oven were mounted (Leco Corp., USA). Resistively heated air was used as a medium for (two) hot jets, while (two) cold jets were supplied by gaseous nitrogen cooled by liquid nitrogen.

An orthogonal gas chromatographic system consisting of a DB-5ms capillary column (30 m length × 0.25 mm I.D. × 0.25 μm film thickness (Agilent, USA)) coupled to a SUPELCOWAX 10 capillary column (1.25 m × 0.10 mm I.D. × 0.10 μm film thickness (Supelco, USA)) was operated under following conditions: primary oven temperature program: 45 °C (0.2 min), 10 °C min^{-1} to 200 °C, 30 °C min^{-1} to 245 °C (1.80 min); secondary oven temperature: +20 °C above the primary oven temperature; modulator offset: +35 °C above the primary oven temperature; modulation period: 3 s (hot pulse 0.6 s); carrier gas: helium; column flow: 1.3 mL min^{-1}. The conditions of TOFMS were as follows: electron ionisation mode (70 eV); ion source temperature: 220 °C; mass range: m/z 25–300; acquisition rate: 300 spectra/s; detector voltage: −1750 V.

ChromaTOF (LECO Corp.) software (v. 2.31) was used for instrument control, data acquisition, and data processing. Compounds identification was based on NIST 2002 mass spectra library search.

2.5. Essential oil and moisture determination

Determination of essential oil content in powdered dried basil samples was performed using a steam-distillation process carried out in a special all-glass apparatus according to the ISO 6571:1984 standard. Dried basil (25 g) was placed into a 0.5 L round-bottom flask together with 250 mL of distilled water. One mL of xylene was added using the side

arm of the apparatus. Within 5 h of distillation the essential oil was gradually trapped in xylene. After cooling, the volume of xylene was recorded and the volume of essential oil was calculated.

For determination of sample moisture the distillation method specified in the ISO 939:1980 standard was employed.

3. Results and discussion

The first part of this study was focused on the development of an HS-SPME method applicable for the determination of basil volatiles. The optimised method was used for analyses of five basil cultivars grown under both ecological and conventional farming conditions. The obtained profiles of volatile compounds were compared with the aim to find similarities/differences and to use them for differentiating between the tested basil varieties. The potential changes in volatiles content during storage of basil samples, as well as the influence of drying and freezing processes on volatiles content were evaluated, too.

3.1. Optimisation of SPME method

HS-SPME as an equilibrium extraction technique requires careful optimisation to obtain high sensitivity and good repeatability of determination. The main factors influencing the whole analytical process, i.e. optimal settings of extraction temperature and time, as well as the choice of suitable fibre stationary phase were investigated during the method development stage. The strategy employed for obtaining optimised parameters, using fresh basil leaves as a sample, is discussed in following paragraphs.

In the first step, four commercially available SPME fibres (see Section 2.1) with different selectivity of stationary phase were evaluated with the aim to find the most efficient for extraction of a wide range of basil volatiles. Fresh basil (cultivar I) served as a sample in fibre testing experiments. All HS-SPME extractions were performed at 40 °C with an extraction time of 20 min.

The volatiles profiles obtained by the tested fibres were fairly similar; nevertheless, for subsequent experiments the mixed DVB/CAR/PDMS fibre was used due to the highest extraction efficiency.

Although the extraction of the basil sample at 40 °C provided higher amounts of volatiles compared to the sorption at 30 °C, to avoid risk of analyte chemical changes, the sorption temperature of 30 °C was chosen for further analyses.

Experiments focusing on the dynamics of basil volatiles extraction were conducted with 1, 5, 10, 20, and 40 min sorption times at 30 °C. Extraction time profiles of eight selected volatile compounds, indicated impossibility to attain the equilibrium for all of them even within the longest sorption time used (40 min). As a compromise between sufficient sensitivity and the speed of analysis, the sorption time of 5 min was used within all following experiments.

3.2. Stability of volatiles in fresh basil samples

While in some studies dealing with the determination of volatiles in basil, dried leaves are analysed (Lee et al., 2005; Grayer et al., 2004), fresh material was employed in our experiments to get information on composition of fresh herb aroma and to recognise potential changes (e.g. oxidation of terpenes) in volatiles profile occurring as a result of the drying process.

Since the preparation of a representative sample for the SPME analysis involves cutting leaves into smaller pieces, the enzymatic processes can, theoretically, take place in injured tissues causing the change of volatiles pattern. Consequently, the time period between sample cutting and sample analysis might become a critical factor, causing distorted results if not properly controlled. To determine the influence of the extraction "dwell time", i.e. the time-delay between the sample preparation and the beginning of its analysis, the repeatability of the method in two sets of samples (A and B) was tested. All the samples belonging to set A were prepared (i.e. plucked, weighed, and capped in the vial) at the same time before each one was sequentially analysed according to the setup of the automated SPME sequence; the dwell time being dependent on the position of a sample in the sequence (ranging from 0 to 150 min). Because the samples in set B were plucked, weighted, and analysed subsequently, the dwell time was reduced to a minimum (no more than 5 min).

The repeatability of the method was determined by performing five replicate analyses in both sets of samples (A and B), as well as, for comparison purposes, by performing five replicate analyses of dried basil sample. RSD values calculated for eight representative compounds are summarised in Table 1.

RSD values (%) obtained for both sets of samples (A and B) were comparable for almost all the compounds evaluated. The only exception was eugenol, in which case the RSD value within set A was as high as 90%. However its RSD value dropped to 22% in the set B, which samples were analysed immediately after preparation. Under such

Table 1
Repeatability of HS-SPME–GC–ITMS method ($n = 5$, fresh basil, DVB/CAR/PDMS 50/30 μm fibre, sorption 5 min at 30 °C)

Selected compounds	Fresh leaves RSD (%), set A	Fresh leaves RSD (%), set B	Dried leaves RSD (%)
α-Pinene	19	21	8
β-Pinene	26	25	10
D-Limonen	19	22	6
1,8-Cineole	20	19	7
Linalool	13	15	5
Camphor	26	21	5
Methyl chavicol	20	19	9
Eugenol	90	22	8

conditions, acceptable RSD values were obtained (15–25%). To achieve this performance characteristic, all samples of fresh basil used in subsequent experiments were analysed immediately after plucking.

The lowest RSD values (5–10%) were obtained in the case of dried basil, proving that dry basil is a more "stabilised" material than fresh basil.

3.3. Identification of compounds

3.3.1. GC–ITMS analysis

As mentioned in Section 2.4.1, a Segment Scan mode was used to enhance the signal of analytes and thus improve the sensitivity of their detection.

Tentative identification of volatile compounds was based on the comparison of measured mass spectra with spectra stored in the library NIST 1.7. Being aware of the fact that retention indices are not available for HP-VOC column used in our study, the commercial standards of major volatiles were used to confirm identification of analytes. The following reference substances were used: β-myrcene, α-humulene, 3-carene, terpinen-4-ol, bornyl acetate, caryophyllene, camphene, D-limonene, α-pinene, and β-pinene.

More than 60 compounds isolated by the HS-SPME method were detected by GC–ITMS, of which 23 were identified. In agreement with some studies published (Jirovetz et al., 2003; Lee et al., 2005), linalool, methyl chavicol, eugenol, bergamotene, and methyl cinnamate represented the dominant volatile components in all analysed basil samples. The list of identified compounds is shown in Table 2.

3.3.2. GC×GC–TOFMS analysis

In the final phase of our experiments, comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC–TOFMS) was used for examination of basil samples. There are two main advantages of GC×GC compared to conventional one-dimensional GC (Dallüge, Beens, & Brinkman, 2003): (i) higher peak capacity as a result of combination of two GC columns with different (independent) separation mechanisms; and (ii) enhanced detectability due to the analyte re-focusing in a modulator and improved separation of chemical noise. Because of these unique features, we decided to explore the potential of this instrumentation in the analysis of volatiles isolated from basil samples.

Fig. 1 illustrates the 3D GC×GC chromatograms of basil volatiles isolated from conventionally and ecologically cultivated cultivar III. Among them, linalool, methyl chavicol, eugenol, and 1,8-cineole were the dominating compounds identified in both analysed samples.

It is worth to notice that thanks to the enhanced detectability provided by this unique technique, we managed to identify several minor volatiles (e.g. phenylethyl alcohol, benzaldehyde, 1-octen-3-ol, fenchyl acetate, (*E*,*E*) 2,4-heptadienal, carveol, geraniol acetate, and others) that were not

Table 2
Volatile compounds identified in fresh basil expressed as a relative percentage ($n = 3$); 100% is equivalent to the sum of all 23 identified compounds

Cultivar	t_R (min)	Cultivar I		Cultivar II		Cultivar III		Cultivar IV		Cultivar V	
Farming[a]		E	C	E	C	E	C	E	C	E	C
Compound		Relative percentage %									
4-Hexen-1-ol	10.26	t	t	t	t	1.2	t	0.2	0.4	0.2	0.4
2-Hexenal	10.28	0.3	0.4	t	t	1.1	t	t	t	1.0	1.0
α-Pinene	11.30	0.6	0.4	1	t	t	t	0.5	0.5	1.1	1.3
Camphene	11.52	t	t	t	t	t	t	t	t	t	t
β-Myrcene	11.99	5.1	4.4	1.1	1.2	1.0	1.2	5.1	5.3	7.0	5.2
β-Pinene	12.05	t	t	1.0	t	t	t	t	t	2.2	2.1
α-Phellandrene	12.35	t	t	t	t	t	t	t	t	t	t
3-Carene	12.48	1.5	1.2	t	t	t	t	t	t	t	t
D-Limonene	12.80	**11.1**	**11. 1**	**15.0**	**12.2**	**11.2**	**7.3**	**3.1**	**3.1**	**2.0**	**1.3**
1,8-Cineole	12.98	**15.6**	**16.9**	**7.1**	**9.2**	**3.1**	**8.2**	**18.1**	**17.3**	**20.0**	**20.2**
Terpinen-4-acetat	13.03	t	t	3.1	4.2	1.0	1.0	t	t	t	t
Linalool	13.61	**15.6**	**18.2**	**24.8**	**32.2**	**21.1**	**17.2**	**23.1**	**26.2**	**21.0**	**19.3**
Camphor	14.93	2.3	t	2.0	t	2.0	1.6	4.2	t	t	t
γ-Terpinene	15.03	0.9	t	2.1	2.0	1.1	1.2	t	t	t	t
Methyl chavicol	15.32	**0.9**	**1.2**	t	t	**10.1**	**44.2**	**2.1**	**1.4**	**1.2**	**1.4**
Bornyl acetate	16.81	**3.2**	**2.2**	**8.2**	**6.2**	**1.2**	**1.1**	t	t	**12.1**	**9.3**
Methyl cinnamate	18.09	ND	ND	ND	ND	**10.2**	ND	ND	ND	ND	ND
Eugenol	19.29	**10.8**	**13.1**	**13.1**	**12.2**	t	t	**22.2**	**17.1**	**12.2**	**9.1**
Bergamotene	19.56	**14.0**	**13.2**	**1.1**	**1.2**	**6.2**	**1.0**	**4.2**	**6.1**	**17.1**	**20.2**
β-Caryophyllene	20.48	2.6	2.8	8.8	10.1	3.2	4.4	6.2	9.1	2.3	3.0
α-Humulene	20.98	t	t	t	t	t	t	t	t	t	t
β-Muurolene	21.16	7.2	6.8	t	t	6.1	6.0	4.3	6.1	3.2	4.0
Cadina-3,9-dien	23.75	t	t	t	t	t	t	t	t	t	t

t – traces.
ND – not detected.
[a] E – ecological cultivation, C – conventional cultivation.

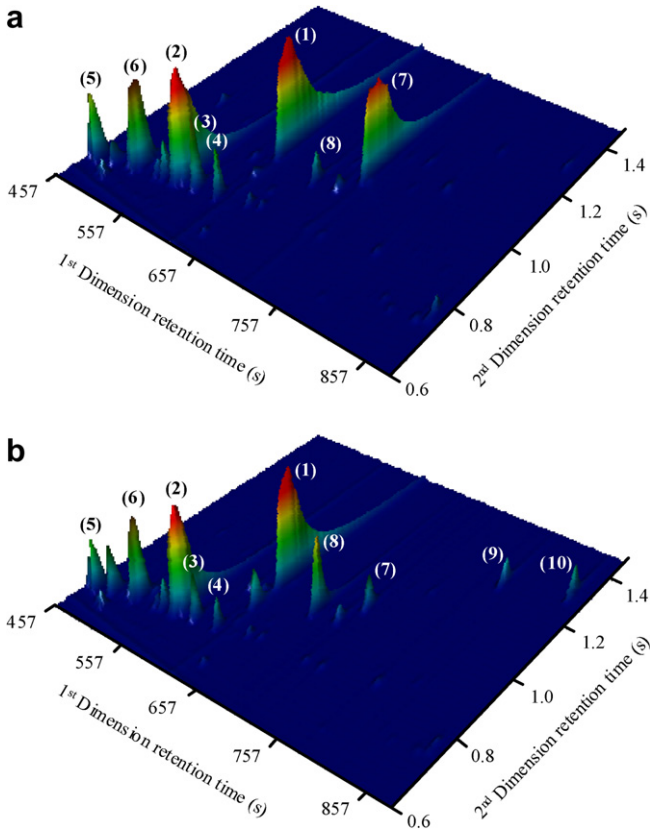

Fig. 1. GC×GC–TOFMS chromatograms of volatiles isolated by SPME from (a) conventional (cultivar III), and (b) ecological (cultivar III). Marked compounds: (1) linalool, (2) 1,8-cineole, (3) γ-terpinene, (4) terpinen-4-acetat, (5) α-pinene, (6) D-limonen, (7) methyl chavicol, (8) camphor, (9) and (10) methyl cinnamate isomers.

detected in a conventional GC–ITMS run. We believe that in close future a GC×GC–TOFMS technique will become a powerful tool in flavour research as indicated in recent studies (Čajka & Hajšlová, 2007; Čajka, Hajšlová, Cochran, Holadová, & Klimánková, 2007).

3.4. Variability of volatiles

3.4.1. Effect of cultivars

Volatile compounds were analysed in five different cultivars of *O. basilicum* L. (see Section 2.2), grown under both organic and conventional conditions. The list of all compounds identified, as well as their relative amounts in each cultivar, is presented in Table 2. The relative content of each particular component is expressed as a relative percentage, being calculated on the basis that 100% is equivalent to the sum of all 23 identified compounds.

Almost all cultivars showed a similar pattern of volatile compounds emitted. The relative amount of major components represented by linalool, eugenol, 1,8-cineole, and bergamotene were in the range of 16–32%, 9–22%, 3–20%, and 1–20%, respectively.

In general, each cultivar had a characteristic aroma profile. The differences between cultivars become obvious when taking into account the pattern of all the major compounds. While in the "red" basils (cultivar III, IV, and V) the amounts of bergamotene, eugenol, and bornyl acetate varied, the "green" cultivars (I and II) differed mainly in content of bergamotene, β-caryophyllene, 1,8-cineole, and methyl chavicol.

Within all basil cultivars, cultivar III was distinguished by higher content of methyl chavicol and absence (not detected) of eugenol. Additionally, methyl cinnamate was not found in any of the cultivars apart from ecologically farmed cultivar III.

3.4.2. Effect of the way of farming

Some morphological differences between "conventionally" and "ecologically" grown plants were observed, the conventionally grown plants being significantly smaller. As also documented in Table 2, the chemical composition, i.e. volatiles profile, was not distinctly affected by the way of farming. The only remarkable exception mentioned earlier was cultivar III, in which case a higher emission of methyl chavicol and an absence of methyl cinnamate were observed in conventionally cultivated plants. This was probably the main cause of a significant difference between the aroma of conventionally and ecologically grown cultivar III.

SPME coupled to GC×GC–TOFMS proved the existence of a difference between conventionally and ecologically farmed cultivar III. The main compounds identified employing this technique are shown in Fig. 1.

3.4.3. Different parts of the plant

To learn more on the volatiles emission by different parts of basil plants, the leaves, haulm, and blossom of cultivar I were collected and immediately analysed using the SPME–GC–ITMS procedure (three repeated analyses were performed).

While the volatiles pattern, see Fig. 2, emitted by blossom and leaves, was quite similar with linalool as one of the major components, 1,8-cineole dominated in haulm.

The overall emissions of volatiles from leaves and blossom were very similar, while the volatiles content in haulm head-space was more than 20 times lower compared with other basil parts.

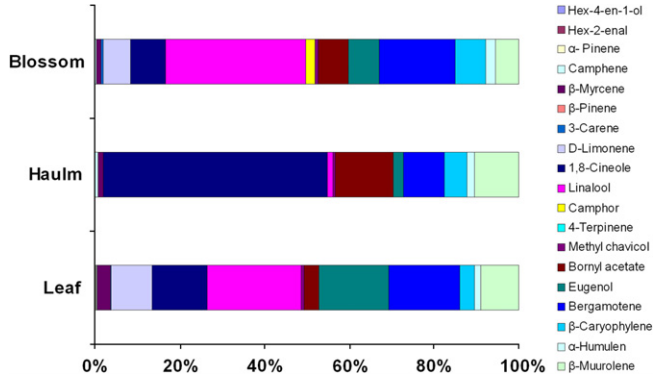

Fig. 2. Relative content ($n = 3$) of volatiles emitted by different parts of basil (cultivar I).

3.4.4. Effect of drying and freezing process

Various physico-chemical changes of aromatic volatiles (oxidation, evaporation) may take place during the drying process, influencing aroma intensity and the quality of the dried product (Barbieri, Elustondo, & Urbicain, 2004; Jerkovic, Mastelic, & Milos, 2001).

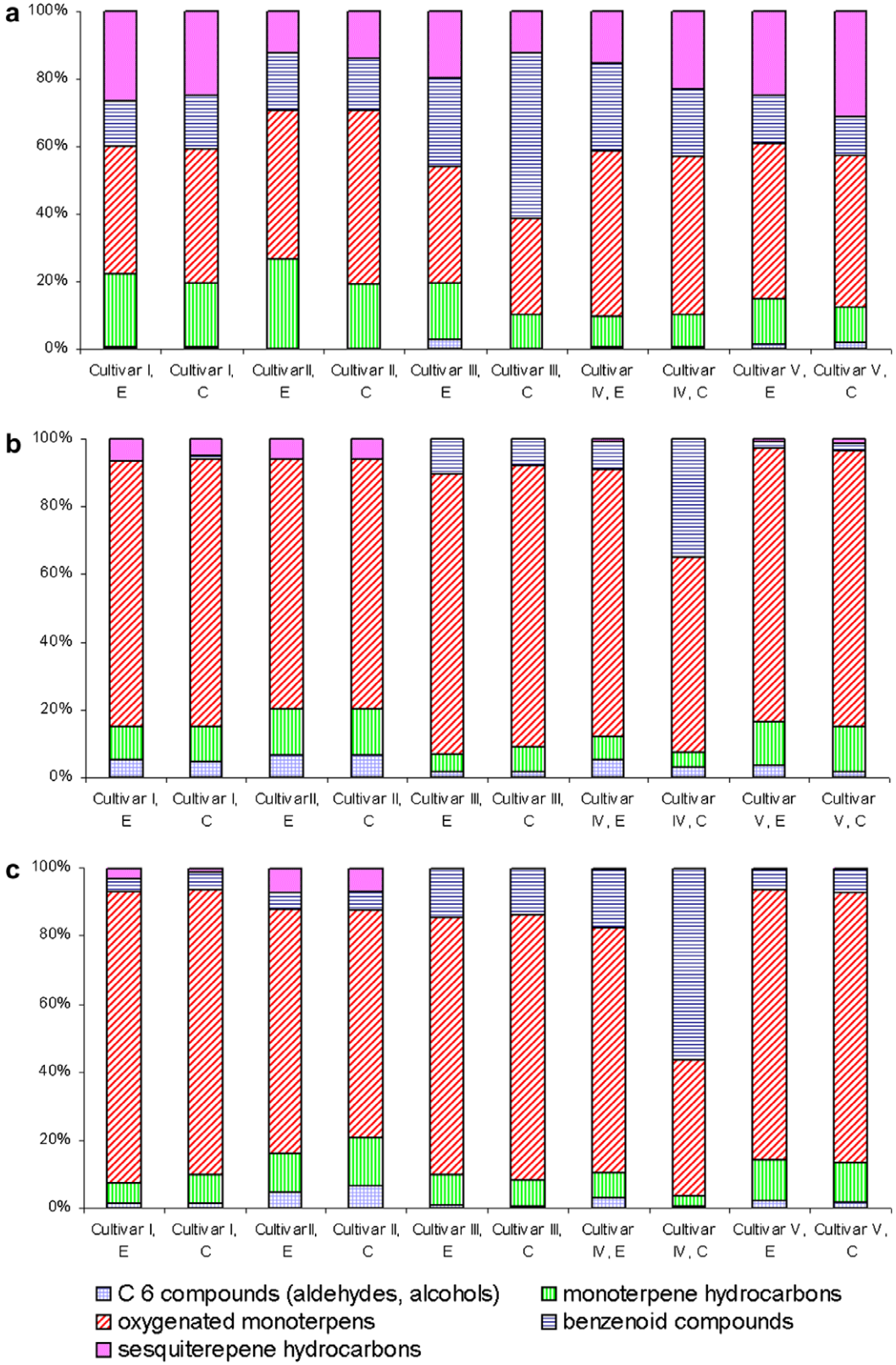

Fig. 3. (a) Relative contents ($n = 3$) of volatiles emitted by fresh leaves. (b) Relative contents ($n = 3$) of volatiles emitted by dried leaves. (c) Relative contents ($n = 3$) of volatiles emitted by frozen leaves.

Table 3
Essential oil and moisture content in fresh and dried basil, ($n = 3$)

Basil cultivar	Farming	Moisture in basil (%) (mean ± s.d.)		Content of essentials oil mL/100 g (mean ± s.d.)
		Fresh	Dried	
Cultivar I	Ecological	11.80 ± 0.54	5.58 ± 0.28	0.80 ± 0.02
	Conventional	11.60 ± 0.48	5.34 ± 0.20	0.70 ± 0.05
Cultivar II	Ecological	11.20 ± 0.45	5.31 ± 0.20	0.71 ± 0.02
	Conventional	11.70 ± 0.61	5.60 ± 0.28	0.89 ± 0.02
Cultivar III	Ecological	12.00 ± 0.54	5.48 ± 0.24	1.10 ± 0.02
	Conventional	11.80 ± 0.55	5.60 ± 0.30	1.11 ± 0.03
Cultivar IV	Ecological	12.30 ± 0.40	5.29 ± 0.35	0.85 ± 0.03
	Conventional	12.00 ± 0.44	5.40 ± 0.28	0.65 ± 0.03
Cultivar V	Ecological	11.90 ± 0.28	5.80 ± 0.34	0.40 ± 0.05
	Conventional	11.90 ± 0.40	5.61 ± 0.38	0.54 ± 0.04

Compared with fresh leaves, a lower amount of extracted volatiles was found in dried and frozen leaves, mainly as a result of the loss of sesquiterpenes, hydrocarbons, benzenoic compounds, and monoterpene hydrocarbons.

The relative contents of volatiles in fresh, dried, and frozen leaves are shown in Fig. 3a–c. Generally, in dried and frozen basil, a decrease in sesquiterpene hydrocarbons (bergamotene, β-caryofyllen, α-humulene), benzenoid compounds (methyl chavicol), and monoterpene hydrocarbons (α-pinene, myrcen, D-limonene) was accompanied by an increase in oxygenated products (1,8-cineole, linalool, and camphor).

3.5. Essential oil and water content

The total content of essential oil in dried basil leaves was determined by means of steam distillation. The obtained results expressed in mL per 100 g are summarised in Table 3.

In agreement with a sensory assessment (data not shown), in which cultivar III was evaluated as the one with the most intensive aroma, this cultivar contained the highest amount of essential oil. Similarly, the sensory panel evaluated the aroma of cultivar V, which contained the lowest amount of essential oil, as the least intensive.

The water content was measured for fresh and dried basil, to found possible differences between samples. The moisture levels were very similar within the groups analysed (see Table 3). Assuming that the storage conditions used (tide plastic bags) avoid significant moisture changes, we did not measure the water content of frozen basil.

4. Conclusions

Automated SPME–GC–ITMS procedure developed in this study enables fast analysis of volatile compounds emitted by basil plants (*O. basilicum* L.). Overlaying head-space volatiles profile (chromatograms), differentiation between five cultivars examined within this study was possible. Linalool, eugenol, 1,8-cineole, and bergamotene were the major volatiles in all the basil samples, their relative abundances, expressed in %, were in the range 16–32, 9–22, 3–20 and 1–20, respectively. Comparing particular cultivars grown under organic or conventional farming conditions, with the exception of cultivar III, no significant differences were found in volatile components sampled by SPME. Interestingly, significantly lower emission of methyl chavicol and the presence of methyl cinnamate were observed in organically grown cultivar III. The results of instrumental analysis were supported by sensory evaluation (data not shown), the cultivar III was evaluated as the one with the most intensive aroma.

We are aware that any general conclusion should by preferable made on the basis of the results of long-term (several years) studies to obtain a stronger statistical support. Nevertheless, the results obtained at least indicate the possible differences between analysed samples.

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