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Research Article

Headspace solid-phase microextraction combined with mass spectrometry as a powerful analytical tool for profiling the terpenoid metabolomic pattern of hop-essential oil derived from *Saaz* variety

Hop (*Humulus lupulus* L., *Cannabaceae* family) is prized for its essential oil contents, used in beer production and, more recently, in biological and pharmacological applications. In this work, a method involving headspace solid-phase microextraction and gas chromatography–mass spectrometry was developed and optimized to establish the terpenoid (monoterpenes and sesquiterpenes) metabolomic pattern of hop-essential oil derived from *Saaz* variety as a mean to explore this matrix as a powerful biological source for newer, more selective, biodegradable and naturally produced antimicrobial and antioxidant compounds. Different parameters affecting terpenoid metabolites extraction by headspace solid-phase microextraction were considered and optimized: type of fiber coatings, extraction temperature, extraction time, ionic strength, and sample agitation. In the optimized method, analytes were extracted for 30 min at 40°C in the sample headspace with a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane coating fiber. The methodology allowed the identification of a total of 27 terpenoid metabolites, representing 92.5% of the total *Saaz* hop-essential oil volatile terpenoid composition. The headspace composition was dominated by monoterpenes (56.1%, 13 compounds), sesquiterpenes (34.9%, 10), oxygenated monoterpenes (1.41%, 3), and hemiterpenes (0.04%, 1) some of which can probably contribute to the hop of *Saaz* variety aroma. Mass spectrometry analysis revealed that the main metabolites are the monoterpene β-myrcene ($53.0 \pm 1.1\%$ of the total volatile fraction), and the cyclic sesquiterpenes, α-humulene ($16.6 \pm 0.8\%$), and β-caryophyllene ($14.7 \pm 0.4\%$), which together represent about 80% of the total volatile fraction from the hop-essential oil. These findings suggest that this matrix can be explored as a powerful biosource of terpenoid metabolites.

Keywords: Essential oil / GC-qMS / Hop *Saaz* variety / HS-SPME / Terpenoid metabolites
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1 Introduction

It is well known that plant-derived natural products are extensively used as biologically active compounds. From these, the essential oils, which represent a small fraction of a plant's composition, and some of their constituents are used not only in pharmaceutical products for their therapeutic activities but also in agriculture, as food preservers and additives for human or animal use, in cosmetics and perfumes, and other industrial fields [1, 2]. Particular emphasis has been placed on their antibacterial, antifungal, and insecticidal activities [3]. In many cases, they serve as (i) plant defense mechanisms against predation by microorganisms, insects, and herbivores; (ii) metal transporting agents; (iii) agents of symbiosis between microbes and plants, nematodes, insects, and higher animals; (iv) sexual hormones; and (v) differentiation effectors [4,5]. Levels of secondary metabolites in plants are both environmentally induced as well as genetically controlled [6].

Among the thousands of metabolites produced by plants, terpenoids represent, by far, the largest and the most diverse class of secondary metabolites, followed by alkaloids and phenolic compounds [7]. They have received special attention by the scientific community, due to their useful and wide range of biological and pharmacological activities [2,8]. Some monoterpenes, such as α-pinene, cineole, eugenol, limonene, terpinolene, citronellol, citronellal, camphor, and thymol, are common constituents of a number of essential oils described

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Abbreviations: HS-SPME, headspace solid phase microextraction; **RI**, retention index

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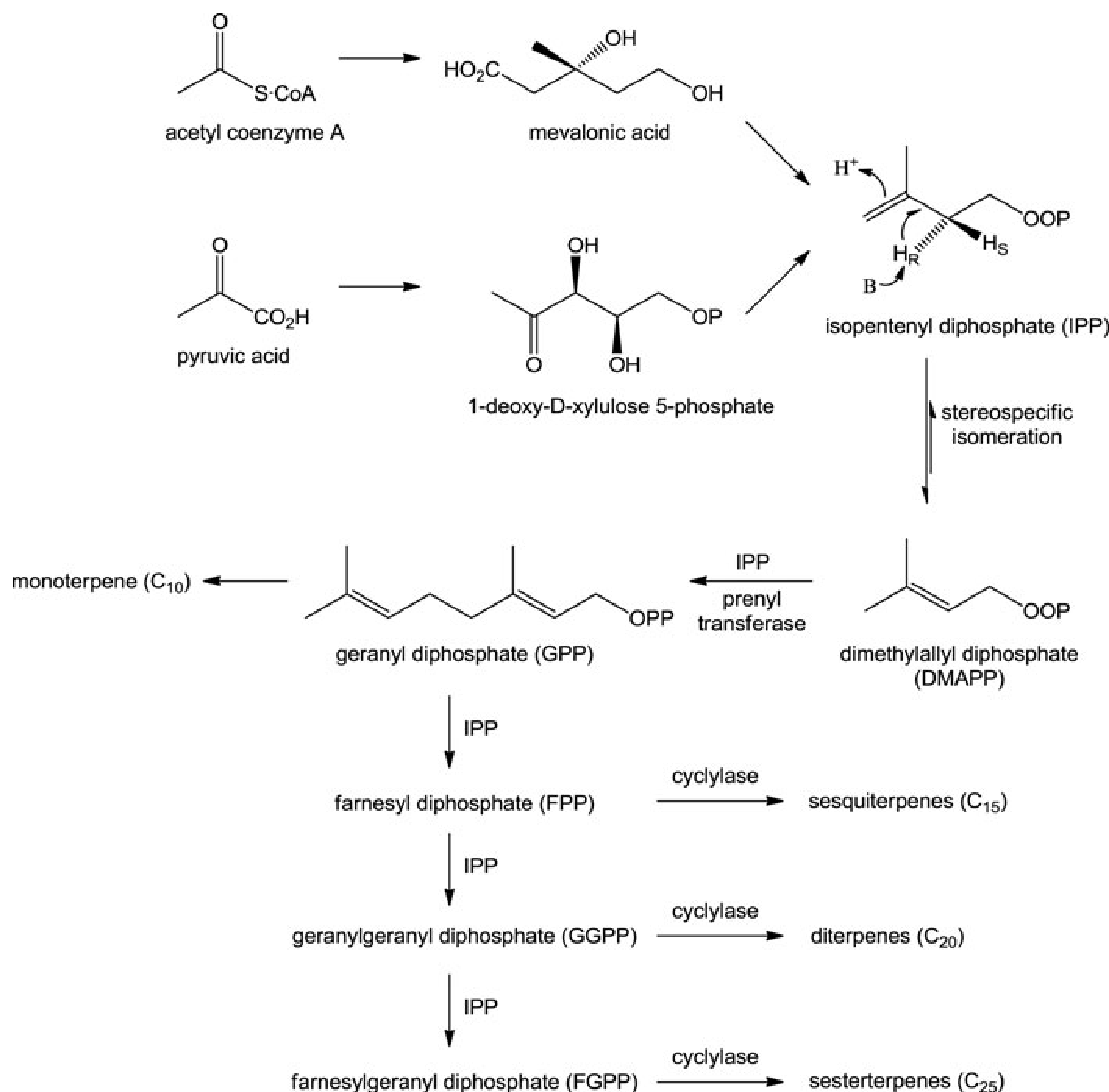

Figure 1. Biosynthetic pathways of terpenoid metabolites [2].

in the literature, as presenting mosquito repellent activity [9]. Among sesquiterpenes, β-caryophyllene is most cited as a strong repellent against *Aedes aegypti* [10]. Although repellent properties of several essential oil regularly appear to be associated with the presence of monoterpenes and sesquiterpenes [9, 11], other authors have found that farnesol has a wide spectrum of desirable biological properties including antitumor [12, 13], antioxidant [14], antifungal, and antibacterial effects [15]. Moreover, farnesol has been demonstrated to selectively inhibit monoamine oxidase B of rat brain [16], a possible role for farnesol in prevention of Parkinson disease [3]. Some others isoprenoids show antiviral (e.g. saponin and glycyrrhizin) [17], antihyperglycemic (e.g. stevioside) [18], anti-inflammatory (e.g. linalool) [19], and antiparasitic (e.g. artemisinin) [20] activities. The biosynthesis of the terpenoid compounds in the essential oil uses the same building blocks as required for the isoprenyl side chains of the hop resins (Fig. 1).

Essential oils represent a small fraction of the composition of plants but confer the characteristics for which aromatic plants are used in the pharmaceutical, food, cosmetic, and fragrance industries [21]. Are complex mixtures containing from a few dozen to several hundred volatile organic compounds produced as secondary metabolites in plants: they are constituted by hydrocarbons (monoterpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols, and phenol ethers) [3]. Their composition may vary considerably between aromatic plant species and varieties, and within the same variety from different geographic areas [22]. Frequently, both hydrocarbons and oxygenated compounds are responsible for the distinctive characteristic odors and flavors of plants.

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Table 1. Summary composition of the most representative commercial hop varieties [23, 24]

Hop variety	Origin	Brewing usage	% α-acids	% β-acids	% Co-humulene[a)]	Total oil (mL/100 g)	% Myrcene[b)]	% Humulene[b)]	% Caryophyllene[b)]	% Farnesene[b)]	Aroma
Ahtanum	USA	Aroma	5.7–6.3	5.0–6.5	30–35	0.8–1.2	50–55	16–20	9–12	<1	Floral, citrus
Brewer's Gold	England	Bittering	5.0–9.0	2.5–3.5	40–48	1.8–2.2	26–41	24–32	4–9	<1	Black currant, fruity, spicy
Cascade	USA	Aroma	4.5–7.0	4.5–7.0	33–40	0.8–1.5	45–60	10–16	3–6	4–8	Medium intensity, floral, citrus, and grapefruit
Centennial	USA	DP[c)]	9.5–11.5	3.5–4.5	28–30	1.5–2.5	45–60	10–18	4–8	<1	Medium intensity, floral, and citrus tones
Chinook	USA	DP	12.0–14.0	3.0–4.0	29–34	1.5–2.5	35–40	20–25	9–11	<1	Medium intensity, spicy, piney, and distinct with subtle tones of grapefruit
Cluster	USA	DP	5.5–8.5	4.5–5.5	36–42	0.4–0.8	45–55	15–18	6–7	0	Strong, floral, and spicy
Crystal	USA	Aroma	3.5–5.5	4.5–7.5	20–26	0.8–2.1	40–60	18–24	4–8	0–1	Mild, floral, and spicy
Fuggle	England	Aroma	4.0–5.5	1.5–2.0	25–32	0.7–1.2	40–50	20–26	6–10	4–5	Mild, woody, and fruity
Galena	USA	DP	12.0–14.0	7.0–9.0	37–42	0.9–1.2	55–60	10–15	3–5	0	Citrus
Golding	England	Aroma	4.0–6.0	2.0–3.0	20–25	0.4–1.0	25–35	35–45	15–20	0	Mild, delicate classic English-type
Hallertau mf	Germany	Aroma	3.5–5.5	3.5–5.5	18–25	0.6–1.5	10–15	36–40	10–12	0	Very mild, slightly flowery, and spicy
Herkules	Germany	Bittering	12.0–17.0	4.0–5.5	32–38	1.6–2.4	30–50	30–45	7–12	<1	Medium intensity, evenly distributed impressions
Hersbrucker	Germany	Aroma	2.0–5.0	2.5–6.0	18–25	0.7–1.3	12.7	32.4	13.6	0	Mild to medium, pleasant, floral, and slightly fruity
Horizon	USA	DP	11–13	6.5–8.5	16–19	1.5–2.0	55–65	11–13	7.5–9.0	2.5–3.5	Floral, spicy
Liberty	USA	Aroma	3.0–5.0	3.0–4.0	24–30	0.6–1.8	32–42	30–40	9–12	0	Mild, slightly spicy
Magnum	Germany	Bittering	10.0–14.0	4.5–7.0	24–30	1.9–3.0	35–45	25–30	8–12	0	No distinct aroma characteristics
Merkur	Germany	Bittering	12.0–15.0	3.5–7.0	16–20	2.2–2.8	48.9	30.7	8.6	0	Strong with earthy, floral, and spicy tones
Millennium	USA	Bittering	14.5–16.5	4.3–5.3	28–32	1.8–2.2	30–40	23–27	9–12	<1	Mild, herbal
Mt. Hood	USA	Aroma	4.0–8.0	5.0–7.5	22–27	1.0–1.3	30–40	25–35	8–15	0	Mild, somewhat pungent
Northern Brewer	England	DP	8.0–10.0	3.0–5.0	20–30	1.5–2.0	50–60	20–30	5–10	0	Medium intensity with Evergreen, wood, and mint overtones
Nugget	USA	Bittering	12.0–14.5	4.0–6.0	24–30	1.7–2.3	52–56	19–20	8–9	0	Mild, herbal, and pleasant
Opal	Germany	Aroma	5.0–8.0	3.5–5.5	13.0–17.0	0.8–1.3	20–45	30–50	8–15	<1	Balanced fruity, hoppy, flowery, citrusy, and herbal characteristics
Palisade™	USA	Aroma	5.5–9.5	6.0–8.0	24–29	1.4–1.6	9–10	19–22	16–18	<1	Floral, fruity, and earthy tones
Perle	Germany	Aroma	7.0–9.5	4.0–5.0	27–32	0.7–0.9	45–55	28–33	10–12	0	Slightly spicy with floral tones
Saaz	Czech Republic	Aroma	2.0–5.0	7.0–8.0	23–28	0.4–1.0	23	20.5	6.0	14.0	Very mild with pleasant hoppy notes

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Table 1. Continued

Hop variety	Origin	Brewing usage	% α-acids	% β-acids	% Co-humulene[a)]	Total oil (mL/100 g)	% Myrcene[b)]	% Humulene[b)]	% Caryophyllene[b)]	% Farnesene[b)]	Aroma
Santiam	USA	Aroma	5.0–7.0	6.0–8.5	20–24	1.3–1.7	27–36	23–26	7–8	13–16	Slightly spicy with herbal and floral tones
Saphir	Germany	Aroma	2.0–4.5	4.0–7.0	12–17	0.8–1.4	~40	~20	~10	0	Distinct aroma with flowery and fruity tones
Smaragd	Germany	Aroma	4.0–6.0	3.5–5.5	13–18	0.7–1.7	20–40	30–50	9–14	<1	Predominantly fruity with hoppy and flowery tones
Spalter	Germany	Aroma	2.5–5.5	3.0–5.0	22–29	0.5–0.9	15.1	25.4	14.6	0	Mild and pleasant with flowery, fruity, and spicy tones
Spalter Select	Germany	Aroma	3.5–5.5	3.5–4.5	20–25	0.8–1.2	40–50	6–8	15–20	10–15	Aroma similar to Spalter hop
Sterling	USA	Aroma	6.0–9.0	4.0–6.0	22–28	1.3–1.9	44–48	6–8	20–22	13–15	Herbal and spicy with a hint of floral and citrus
Strisselspalt	France	Aroma	3.0–5.0	3.0–5.5	20–25	0.6–0.9	n.i.	28–32	n.i.	n.i.	Medium intensity, pleasant, and hoppy
Taurus	Germany	Bittering	12.0–17.0	4.0–6.0	20–25	0.9–1.4	30	30	8.4	0.2	Strong
Tettnanger	Germany	Aroma	4.0–5.0	3.0–4.5	20–25	0.4–0.8	36–45	18–23	6–7	10–12	Slightly spicy
Tomahawk®	USA	Bittering	14.0–18.0	4.5–5.8	29–34	2.0–3.5	25–40	10–22	7–12	0	Earthy, spicy, pungent, with some citrus overtones
Tradition	Germany	Aroma	5–7	4–5	26–29	1.0–1.4	21.8	48.4	13.4	<0.1	Medium intensity, floral, and herbal tones
Ultra	USA	Aroma	4.0–5.0	3.6–4.7	25–30	0.8–1.2	25–35	30–40	10–15	0	Similar aroma profile to Hallertauer Mittelfrüher
Willamette	USA	Aroma	4.0–6.0	3.0–4.5	30–35	1.0–1.5	20–30	20–30	8–12	5–10	Mild and pleasant, slightly spicy

a) Percentage relatively to α-acids.
b) Percentage relatively to total oil.
c) DP: Dual purpose: Used for its aromatic properties and bittering potential.
d) n.i.: No information.

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Over centuries, hop (*Humulus lupulus* L.) was used primarily as an essential ingredient in the manufacturing of beer since its components add the typical bitter taste and contribute to the attractive aroma of the final beverage. Essential oil of hop comprises two major fractions: the first belongs to the group of hydrocarbons of which terpene hydrocarbons account for about 70% [25]. The remaining 30% are compounds containing oxygen (oxygenated fraction that is generally more aromatic and less volatile) such as esters, aldehydes, ketones, acids, and alcohols [3]. Different hop varieties produce different essential oils that can have widely distinct taste, odor, and aroma, depending on their chemical nature (Table 1).

Geographical location, climate, and agronomical factors also affect the oil composition, potentially creating different profiles for hop samples with the same genetic material [3].

In recent years, essential oil has received much attention as potentially useful bioactive compounds against insects. Although effective, the constant application of pesticides to control insects, can disrupt the natural biological control systems and has led to outbreaks of insect species, which sometimes resulted in the widespread development of resistance, had undesirable effects on nontarget organisms, and fostered environmental and human health concerns. These problems have highlighted the need for the development of new strategies for selective and specific pest control. Furthermore, the different activities of aromatic plants essential oils, such as antimicrobial, antiviral, and anticarcinogenic activities, explain their broad use in phytotherapy [26]. Particularly, the antimicrobial activity has formed the basis of many applications, including raw and processed food preservation, pharmaceutical, alternative medicine, and natural therapies. According to Bozin et al. [26], this aspect assumes a unique relevance due to an increased resistance of some bacterial strains to the most common antibiotics and antimicrobial agents for food preservation.

A range of extraction and concentration methods have been developed for the analysis of essential oil, which include steam distillation [27] or extraction with a conventional solvent [28], supercritical fluid CO_2 extraction [29], column chromatography [30], and stir bar sorptive extraction [31]. Nevertheless, these techniques present certain nonnegligible drawbacks such as the use of high volumes of solvent, the time required, and the use of expensive devices with a limited lifetime that may entail carry-over or cross-contamination problems. Consequently, in order to overcome these drawbacks, solid-phase microextraction (SPME) has emerged as an efficient extraction-preconcentration method and a reliable alternative to traditional sample preparation techniques, due to important features such as simplicity, low cost, selectivity, and sensitivity when combined with appropriate detection modes [32–37]. This method, developed by Pawliszyn and co-workers [38, 39], eliminates the use of organic solvents, and substantially shortens the time of analysis. SPME can integrate sampling, extraction, concentration, and sample introduction into a single uninterrupted process, resulting in high sample throughput and also be used as a solvent-free sample preparation method with gas chromatography (GC) mass spectrometry (MS) analysis, which has been successfully applied for profiling the metabolomic pattern of fruits [40–44], and analysis of environmental [45], food [40, 42, 44], forensic [46], and pharmaceutical samples [47] and also as a powerful technique for extraction of urinary potential cancer biomarkers [48, 49].

In the present communication, we report on using SPME, in headspace mode (HS-SPME), coupled to GC-qMS (quadrupole first stage mass spectrometry) as a powerful methodology to investigate the metabolomic pattern of terpenoid composition in hop-essential oil derived from *Saaz* variety as a mean to explore, in a near future, these matrix as a powerful biological source of antimicrobial (antibacterial and antifungal) and antioxidant agents, constituting an environmentally friendly alternative as potential substitutes for synthetic compounds. Important SPME experimental parameters that may affect extraction efficiency, namely, nature of fiber coating, extraction temperature, extraction time, ionic strength, and sample agitation, were considered on this study. The optimized conditions were applied to the characterization of the terpernoid metabolites in hop-essential oil from *Saaz* variety. The method is simple, requires small amounts of sample, and was expected to provide global terpenoid metabolomic signature of hop-essential oil while offering a significant time reduction when compared to other methods commonly used.

2 Materials and methods

2.1 Reagents and materials

The SPME holder for manual sampling and the fibers used were purchased from Supelco (Bellefonte, PA, USA). Amber silanized glass vials (4.0 mL) were obtained from Agilent Technologies (Palo Alto, CA, USA). According to manufacturer's recommendation, the fibers were first conditioned in the GC injection port to remove fiber contaminants. Prior to extraction, the fiber was, daily, inserted in the hot injection port for 6 min. A blank test was performed to check possible carry-over. The Kovat's retention index (RI) was calculated through injection of a series of C_8–C_{20} straight-chain *n*-alkanes (concentration of 40 mg/L in *n*-hexane) purchased from Fluka (Buchs, Switzerland). Sodium chloride, of analytical grade, was purchased from Panreac Quimica SA (Barcelona, Spain).

2.2 Samples

Five hop-essential oil samples, obtained by supercritical CO_2 extraction, were kindly provided by Empresa de Cervejas da Madeira (ECM), Madeira Island, Portugal. Samples were transported under refrigeration (ca. 2–5°C) to the laboratory and stored at −20°C until analysis. All samples were analyzed in triplicate.

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2.3 HS-SPME extraction conditions

Right before analysis, samples were thawed at 20°C for 10 min and then were subjected to HS-SPME. Extraction was carried out using 0.5 g of hop-essential oil into a 4-mL glass HS vial. The samples were equilibrated during the incubation time (10 min in all assays) in a temperature-controlled six-vial agitator tray at the appropriate temperature and time (selected according to the optimization design). Subsequently, the SPME fiber was manually inserted into the sealed vial through the septum and the fiber was exposed to the sample HS for a specific extraction time and extraction temperature. Following the extraction process, the fiber was retracted prior to remove from the sample vial and immediately inserted into the GC-qMS injector for thermal desorption of metabolites at 250°C for 6 min in splitless mode. All measurements were made with, at least, three replicates.

2.4 Optimization of SPME parameters

The effectiveness of analyte preconcentration using the SPME technique depends on several experimental parameters, from which the fiber coating, extraction time, and extraction temperature are the most significant. For this reason, the extent to which each of these variables affects the efficiency of SPME procedure was examined by application of univariate optimization design.

2.4.1 Selection of the fiber coating

In the preliminary selection, all commercially available silica SPME fibers, varying in polarity, thickness of the stationary phase, and coated with the following polymers: polydimethylsiloxane (PDMS, 100 μm), PDMS/divinylbenzene (PDMS/DVB, 65 μm), DVB/carboxen on PDMS (DBV/CAR/PDMS; StableFlex, 50/30 μm), CAR/PDMS (CAR/PDMS, 75 μm), polyacrylate (PA, 85 μm), and polyethyleneglycol (PEG, 60 μm) were tested in order to select the best polymer to extract the terpenoid metabolites. In this step, all the fibers were exposed to the sample HS under the following conditions: 10 min of equilibrium time, 30 min of extraction time, and 40°C for extraction temperature (conditions arbitrarily established by the authors in the choice-of-fiber step). Fibers were thermally conditioned in accordance with the manufacture's recommendations before first use. Before the first daily analysis, and in order to guarantee the absence of peaks in the run blanks and the good quality of the SPME extraction and chromatographic procedures, each of the fibers was reconditioned at 250°C for 15 min, following the manufacturer's recommendations. All the fibers were tested in triplicate and the results presented represent the mean values obtained.

2.4.2 Effect of extraction time and temperature

Extraction time and temperature are two of the most important parameters affecting the volatility of analytes. Therefore, these two parameters were optimized. The procedure described in Section 2.3 was employed to evaluate the extraction time and temperature. The HS-SPME extraction of the hop-essential oil samples (CO_2 supercritical extract) was done using fiber exposure times between 15 and 60 min using DVB/CAR/PDMS fiber at 40°C. In order to optimize the extraction temperature, up to three consecutive extractions were carried out at each of the following temperatures: room temperature (24°C), 30, 40, and 50°C using DVB/CAR/PDMS fiber for 30 min.

2.5 GC-qMS conditions

The SPME-coating fibers containing the adsorbed terpenoid metabolites extracted from the hop-essential oil were manually introduced into the GC injection port at 250°C and kept for 6 min for desorption. The split/splitless injector, operating in the splitless mode, was equipped with an inlet liner for SPME (internal diameter 0.75 mm i.d., Supelco, Barcelone, Spain). The desorbed terpenoid metabolites were separated in an Agilent Technologies 6890N Network GC equipped with a BP-20 fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) supplied by SGE (Darmstadt, Germany) connected to an Agilent 5973N quadrupole mass selective detector. Helium (Air Liquid, Portugal) was used as carrier gas at 1.1 mL/min constant flow (column head pressure: 12 psi). The injections were performed in the splitless mode (5 min). The GC oven temperature was programmed as follows: 40°C for 1 min, 1.7°C/min ramp until 180°C (1 min) then to 220°C at 30°C/min and held isothermally at 250°C for a further 1 min. For the MS system, the temperatures of the transfer line, quadrupole, and ionization source were 250, 180, and 230 ³C, respectively; electron impact mass spectra were recorded at 70 eV and the ionization current was about 30 μA. Data acquisitions were performed in scanning mode (mass range *m/z* 30–300; 6 scans per second). The GC peak area of each compound was obtained from the ion extraction chromatogram by selecting target ions for each one. Reproducibility was expressed as relative standard deviation (RSD). Signal acquisition and data processing were performed using the HP Chemstation (Agilent Technologies).

Terpenoid metabolites identification was based on (i) comparison of the GC retention time and mass spectra, with those, when available, of the pure standard compounds; (ii) comparison between the MS for each putative compound with those of the data system library (NIST, 2005 software, Mass Spectral Search Program V.2.0d; NIST 2005, Washington, DC, USA); and (iii) Kovat's RI determined according to the Van den Dool and Kratz [50]. For the determination of the RI, a C_8–C_{20} *n*-alkanes series was used, and the values were compared, with available values reported in the literature for

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similar chromatographic columns. All Identity Spectrum Mach factor above 850 resulting from the NIST Identity Spectrum Search algorithm (NIST MS Search 2.0) was determined to be acceptable for positive identification.

Monoterpenes and specially sesquiterpenes are notoriously difficult to resolve and identify because they have the same molecular formulae and therefore interact with column stationary phases in the same manner and exhibit very similar mass spectra.

3 Results and discussion

The influence of the main parameters that can affect the HS-SPME process from HS, i.e. fiber coating, extraction temperature, extraction time, ionic strength, and sample agitation, was evaluated. HS-SPME mode was used instead of direct sampling mode because, for volatile analytes, in the former mode the equilibrium times are shorter compared to direct extraction. The HS mode also protects the fiber from adverse effects caused by nonvolatile, high molecular weight substances present in the sample matrix. Temperature has a significant effect on the extraction kinetics, since it determines the vapor pressure of the analytes, and for that their influence in the extraction process was also investigated. In the optimized method, analytes were absorbed for 30 min at 40°C in the sample HS with a 50/30 μm DVB/CAR/PDMS fiber. The best conditions obtained for HS-SPME/GC-qMS methodology was chosen based on intensity response (GC peak area), number of identified compounds, and RSD (RSD, %). After the optimization step, the terpenoid metabolomics profile of the hop-essential oil derived from *Saaz* variety was established.

3.1 Optimization of HS-SPME parameters

The optimization of the different parameters involved in HS-SPME was performed choosing the conditions that allowed obtaining the maximum response in terms of analyte peak area.

3.1.1 Fiber-coating selection

The selection of a suitable fiber coating is an important step in SPME optimization. The sensitivity of the SPME extraction technique depends greatly on the value of the distribution constant of analytes partitioned between the sample and fiber-coating material. For this reason, six different types of SPME fibers were evaluated in this study, in order to assess that the coating having highest affinity toward terpenoid metabolites. The comparison of the SPME fiber performance was based on extraction efficiency, estimated by total peak area, number of isolated compounds from the extract, and reproducibility. Table 2 reports the results of the relative extraction efficiency of the six SPME fibers with respect to their capacity to extract the terpenoid metabolites of the *Saaz* hop-essential oil. Each fiber was exposed to the HS under the same conditions of equilibrium time (10 min), extraction time (30 min), and temperature (40°C), and although the extraction conditions were the same, the differences in the areas obtained revealed the behavior of each type of coating used for each fiber tested (Table 2). The results of this screening showed that the highest extraction sensitivity was obtained with the CAR-related stationary phase. Although the means of the total areas obtained for the PDMS, DVB/CAR/PDMS, and CAR/PDMS fibers did not present significant statistical differences (Tukey at $P < 0.05$), the fiber DVB/CAR/PDMS was chosen, since it presented the best extraction efficiency for a highest number of terpenoid metabolites (Table 2). The good performances obtained with fibers containing PDMS coating were partially expected since PDMS is a lypophilic coating, so with a higher affinity than the partially polar PA and PEG, for nonpolar molecules such as terpenoid metabolites.

Conversely, the lowest sorption capacity expressed as chromatographic areas ($P < 0.05$) were in general obtained with the PA fiber under the same experimental conditions.

DVB/CAR/PDMS coating (molecular weight ranging from 35 to 300) combines the absorption properties of the liquid polymer with the adsorption properties of porous particles, which contains macro- (>500 Å), meso- (20–500 Å), and microporous (2–20 Å), and has bipolar properties. The mutually synergetic effect of adsorption and absorption of the stationary phase explains its high retention capacity. Based on the data evaluation completed within this particular optimization experiment, DVB/CAR/PDMS fiber was chosen to be used for all further optimization steps and hop-essential oil analysis experiments, without adding salt and without agitation of the sample. Using the DVB/CAR/PDMS 50/30 μm fiber, the addition of salt and the agitation of the sample led to a decrease of chromatographic peak areas ($P < 0.05$) for some analytes. Similar results were obtained by Laura Campo et al. [51] in the quantification of 13 priority polycyclic aromatic hydrocarbons in human urine by HS-SPME GC and isotope dilution MS.

3.1.2 Extraction time and temperature

Extraction time and temperature are very important experimental factors to define the optimum extraction conditions from the HS. Since time affects the mass transfer of the analytes onto the fiber, optimum time is required for the fiber to reach equilibrium with HS. To study the effects of extraction time, *Saaz* hop-essential oil samples were extracted for predetermined extraction times ranging from 15 to 60 min at 40°C. The results are shown in Fig. 2A. A typical extraction time profile consists of an initial rapid portioning followed by a slower prolonged uptake and finally a steady-state equilibrium between the fiber and the vapor phase of the analyte. As can be observed, over 30 min, no significant increase in the response was observed. Moreover, 30 min showed excellent reproducibility (RSD = 1.0%) when compared with 45 (RSD = 8.4%) and 60 min (RSD = 6.8%).

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Table 2. Retention times, literature, and calculated Kovat's retention indices (RI), metabolites identification, *m/z* of major fragment ions, and effect of fiber type on the peak area ($n = 3$) ($\times 10^6$ area units) of terpenoid metabolites from hop-essential oil of *Saaz* variety as determined by HS-SPME/GC-qMS [52]

Peak no.	RT[a] (min)	RI_{lit}[b]	RI_{cal}[c]	Identification[d]	Metabolite	MF[e]	*m/z*[f]	Similarity (%)	Peak area ($\times 10^6$ area units) ± RSD (%)					
									DVB/CAR/PDMS[g]	PDMS[g]	CAR/PDMS[g]	PDMS/DVB[g]	PEG[g]	PA
1	1.42	-	908	MS, RI	Isoprene	C_5H_8	32, 67, 53	96	0.1 ± 7.8	-	0.4 ± 1.2	0.7 ± 1.5	-	-
2	3.92	1007	1016	MS, RI, ST	α-Pinene	$C_{10}H_{16}$	93, 77, 121	93	0.7 ± 9.7	1.0 ± 2.8	0.6 ± 2.9	0.5 ± 2.5	0.1 ± 3.1	0.02 ± 8.2
3	4.77	1075	1057	MS, RI	Camphene	$C_{10}H_{16}$	93, 121, 41	94	0.04 ± 8.7	0.2 ± 9.5	0.1 ± 8.5	0.3 ± 3.2	0.01 ± 5.5	-
4	5.73	1116	1107	MS, RI, ST	β-Pinene	$C_{10}H_{16}$	93, 41, 69	91	2.4 ± 1.2	5.8 ± 3.9	3.0 ± 3.2	3.2 ± 8.0	0.5 ± 2.7	0.1 ± 1.3
5	8.79	1145	1135	MS, RI, ST	β-Myrcene	$C_{10}H_{16}$	93, 69, 41	97	153.3 ± 1.1	191.1 ± 1.7	159.3 ± 1.9	126.7 ± 1.9	27.7 ± 2.5	7.6 ± 5.0
6	9.53	1180	1172	MS, RI, ST	Limonene	$C_{10}H_{16}$	68, 93, 79	93	1.3 ± 1.3	1.1 ± 5.0	1.2 ± 5.0	1.7 ± 7.6	0.3 ± 2.9	0.1 ± 2.1
7	10.01	1185	1182	MS, RI	β-Phellandrene	$C_{10}H_{16}$	93, 77, 136	95	1.1 ± 2.6	0.2 ± 6.8	0.9 ± 5.1	1.1 ± 1.0	-	-
8	11.64	1242	1246	MS, RI	*trans*-β-Ocimene	$C_{10}H_{16}$	93, 79, 41	89	0.1 ± 1.2	0.1 ± 7.4	0.1 ± 1.1	0.1 ± 2.4	0.01 ± 4.2	-
9	11.79	1262	1267	MS, RI, ST	α-Terpinene	$C_{10}H_{16}$	93, 136, 77	91	0.1 ± 1.4	0.1 ± 3.3	0.1 ± 2.6	0.1 ± 2.7	0.01 ± 3.5	-
10	12.48	1274	1279	MS, RI	*cis*-β-Ocimene	$C_{10}H_{16}$	93, 76, 41	92	2.1 ± 1.3	2.6 ± 3.2	2.2 ± 2.5	1.5 ± 2.5	0.2 ± 1.0	0.03 ± 1.3
11	13.15	-	1290	MS, RI, ST	o-Cymene	$C_{10}H_{14}$	119, 32, 134	95	0.1 ± 1.9	0.1 ± 2.7	0.1 ± 1.3	0.04 ± 1.3	0.01 ± 1.2	-
12	13.75	1284	1280	MS, RI	α-Terpinolene	$C_{10}H_{16}$	93, 121, 136	97	0.1 ± 3.4	0.1 ± 3.6	0.1 ± 1.0	0.1 ± 2.8	0.01 ± 1.1	-
13	22.88	1295	1305	MS, RI	Perillene	$C_{10}H_{14}O$	69, 81, 150	96	0.2 ± 1.5	0.2 ± 8.6	0.2 ± 2.9	0.2 ± 2.5	0.03 ± 2.3	-
14	26.07	1476	1481	MS, RI	Ylangene	$C_{15}H_{24}$	105, 119, 161	95	0.4 ± 2.0	0.2 ± 5.8	0.3 ± 2.0	0.2 ± 3.2	-	-
15	26,64	1480	1491	MS, RI	α-Cubebene	$C_{15}H_{24}$	161, 119, 105	91	1.2 ± 2.0	0.8 ± 1.7	0.8 ± 1.6	0.7 ± 3.5	0.1 ± 2.2	0.3 ± 1.6
16	33.62	1663	1621	MS, RI, ST	α-Humulene	$C_{15}H_{24}$	133, 93, 69	96	48.1 ± 7.5	52.8 ± 2.0	41.4 ± 1.5	36.8 ± 3.7	10.9 ± 2.6	3.1 ± 8.0
17	38.63	1657	1665	MS, RI, ST	β-Caryophyllene	$C_{15}H_{24}$	93, 80, 121	93	42.4 ± 4.1	21.3 ± 2.3	16.8 ± 1.9	15.4 ± 3.6	3.6 ± 2.5	1.0 ± 2.2
18	39.90	1681	1687	MS, RI	γ-Muurolene	$C_{15}H_{24}$	161, 105, 119	88	1.7 ± 2.7	1.3 ± 2.8	0.9 ± 1.8	0.8 ± 1.4	0.6 ± 3.3	0.1 ± 1.9
19	41.40	1566	1554	MS, RI	Methyl geranate	$C_{11}H_{18}O_2$	69, 41, 114	90	3.7 ± 1.1	3.1 ± 4.2	1.3 ± 3.1	2.3 ± 2.0	0.4 ± 2.8	0.1 ± 3.2
20	41.89	1724	1713	MS, RI, ST	α-Selinene	$C_{15}H_{24}$	189, 161, 93	92	0.8 ± 9.8	0.2 ± 2.3	0.2 ± 6.8	0.4 ± 4.1	0.2 ± 3.0	0.04 ± 2.3
21	42.50	-	1744	MS, RI	α-Muurolene	$C_{15}H_{24}$	105, 161, 93	89	0.3 ± 2.5	0.3 ± 1.8	0.2 ± 1.6	0.2 ± 4.1	0.1 ± 3.6	0.02 ± 1.4
22	44.59	1749	1740	MS, RI, ST	(+)-δ-Cadinene	$C_{15}H_{24}$	161, 134, 119	89	1.9 ± 2.2	1.6 ± 1.8	1.0 ±1.5	1.0 ± 4.2	0.4 ± 2.6	0.1 ± 1.0
23	45.88	-	1755	MS, RI, ST	Cubenene	$C_{15}H_{24}$	119, 105, 161	93	0.3 ± 5.4	0.2 ± 5.7	0.1 ± 1.2	0.2 ± 4.4	0.1 ± 3.8	0.01 ± 7.1
24	46.60	-	1769	MS, RI	Naphthalene H4,7DM 1R[h]	$C_{15}H_{24}$	105, 161, 91	96	0.2 ± 2.5	0.1 ± 2.1	0.1 ± 2.0	0.1 ± 4.7	0.1 ± 6.3	0.01 ± 9.8
25	48.27	-	1780	MS, RI	β-Fenchene	$C_{10}H_{16}$	79, 32, 67	95	0.1 ± 3.0	0.1 ± 4.6	0.02 ± 2.7	0.04 ± 5.2	0.01 ± 1.1	0.01 ± 1.0
26	49.30	1800	1789	MS, RI, ST	3-Carene	$C_{10}H_{16}$	69, 93, 41	98	0.9 ± 2.5	0.4 ± 1.1	0.3 ± 2.3	0.4 ± 5.0	0.3 ± 1.2	0.04 ± 8.0
27	51.68	1825	1835	MS, RI, ST	*cis*-Geraniol	$C_{10}H_{18}O$	69, 41, 93	92	0.2 ± 2.0	0.1 ± 3.9	0.1 ± 3.2	0.1 ± 2.0	0.1 ± 1.0	-
					Total peak area ($\times 10^6$)				**263.7**	**285.0**	**231.8**	**194.4**	**45.6**	**12.4**
					Average RSD (%)				**3.4**	**3.9**	**2.7**	**3.4**	**2.8**	**3.9**
					No. metabolite by fiber				**27**	**26**	**27**	**27**	**24**	**17**

a) Retention time (min).
b) Kovat's retention index reported in the literature for BP-20 capillary column or equivalents [53].
c) Kovat's retention index relative n-alkanes(C_8–C_{20}) on a BP-20 capillary column.
d) The reliability of the identification or structural proposal is indicated by the following: MS-NIST and Wiley libraries spectra and the literature; RI—Kovat's retention index on a BP-20 capillary column in reference [30]; ST—coinjection with authentic standard compounds.
e) Molecular formula.
f) Ordered by decreasing intensity, being the peak base the fragment on the left side.
g) Mean of triplicate assays ± RSD (relative standard deviation = 100 s/x), where s is the standard deviation of n measurements and x is the mean of the same n measurements
h) 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1methylethyl)-, [1R-(1-alpha, 4a.alpha, 8a.alpha)]-naphthalene.

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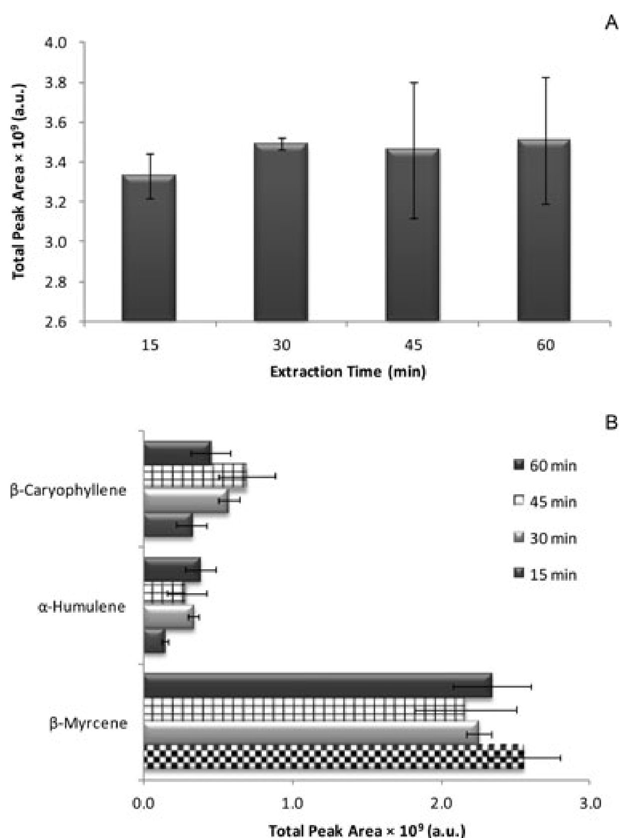

Figure 2. Influence of the extraction time on the extraction efficiency of terpenoid metabolites by HS-SPME (fiber 50/30 μm DVB/CAR/PDMS, extraction temperature of 40°C), expressed as (A) total peak area; and (B) profile of major terpenoid metabolites (a.u. arbitrary units).

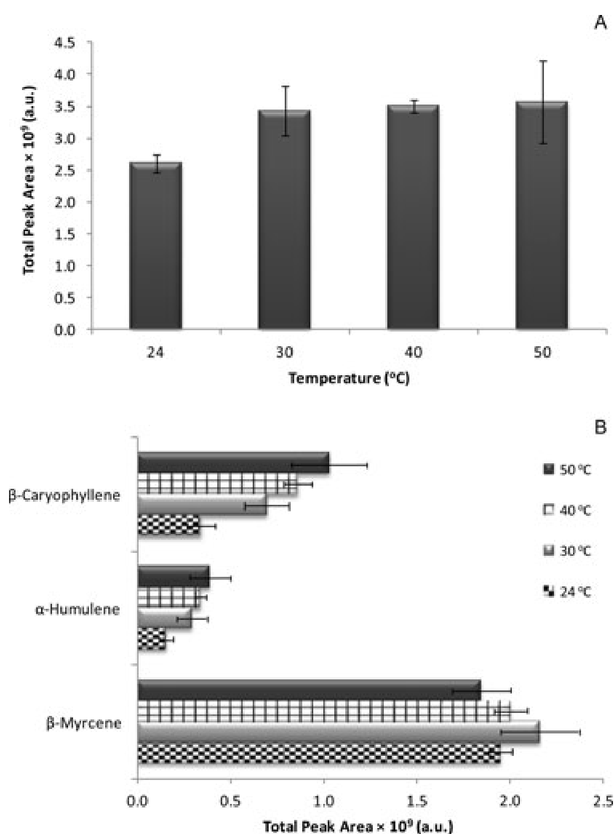

Figure 3. Effect of the extraction temperature on the extraction efficiency of terpenoid metabolites from *Saaz* hop-essential oil by HS-SPME (fiber, 50/30 μm DVB/CAR/PDMS, extraction time, 30 min; (A) total peak area of the terpenoid fraction; and (B) profile of the major terpenoid metabolites (a.u. arbitrary units).

The extraction time profile of the major terpenoid metabolites in essential oil from hop of *Saaz* variety is represented in Fig. 2B. For some analytes, higher chromatographic responses were observed for longer sampling time. The peak area for β-myrcene decreased with time, while α-humulene and β-caryophyllene reach the steady-state equilibrium at 30 and 45 min, respectively. Considering the results for the 27 identified terpenoid metabolites, an extraction time of 30 min was chosen as a good compromise between obtaining an optimized chromatographic signal and a reasonable analysis time. In addition, the lower extraction time can extend the lifetime of the SPME fiber.

The extraction temperature presents several effects on extraction efficiency. The temperature increases diffusion coefficients and Henry's constants while the time required to reach equilibrium decreases [54]. To evaluate the effect of temperature on SPME extraction efficiency, different extraction temperatures (24, 30, 40, and 50°C) were investigated. The results concerning total GC-qMS peak area as a function of temperature are illustrated in Fig. 3A. It can be observed that the extracted amount increases with the increase of the extraction temperature. Increase in extraction temperature will improved the mobility of volatile compounds through liquid and gas phases leading to an increase in extraction amounts. However, increasing temperature over 30°C, no significant increase in the total response was observed. In addition, in what concerns the GC-qMS response (based on peak areas) as a function of temperature (Fig. 3A), a high reproducibility was obtained at 40°C (RSD = 2.9%) in comparison to 30°C (RSD = 11.4%) and 50°C (RSD = 16.4%), respectively; therefore, 40°C was selected as extraction temperature for further studies.

The absorption kinetics at different temperature of the most abundant terpenoid metabolites absorption found in hop-essential oil is shown in Fig. 3B. As well for extraction time, and taking into account the three major compounds (β-myrcene, α-humulene, and β-caryophyllene), it can be observed that an extraction temperature of 30°C afforded the highest extraction sorption for β-myrcene, in contrast to α-humulene and β-caryophyllene that extraction efficiency was highest when the HS-SPME extraction was performed at 50°C. From these findings, an absorption temperature of 40°C was chosen in order to maximize the analytical response of all compounds.

The conditions selected as optimal for the establishment of the terpenoid metabolomics pattern from hop-essential oil

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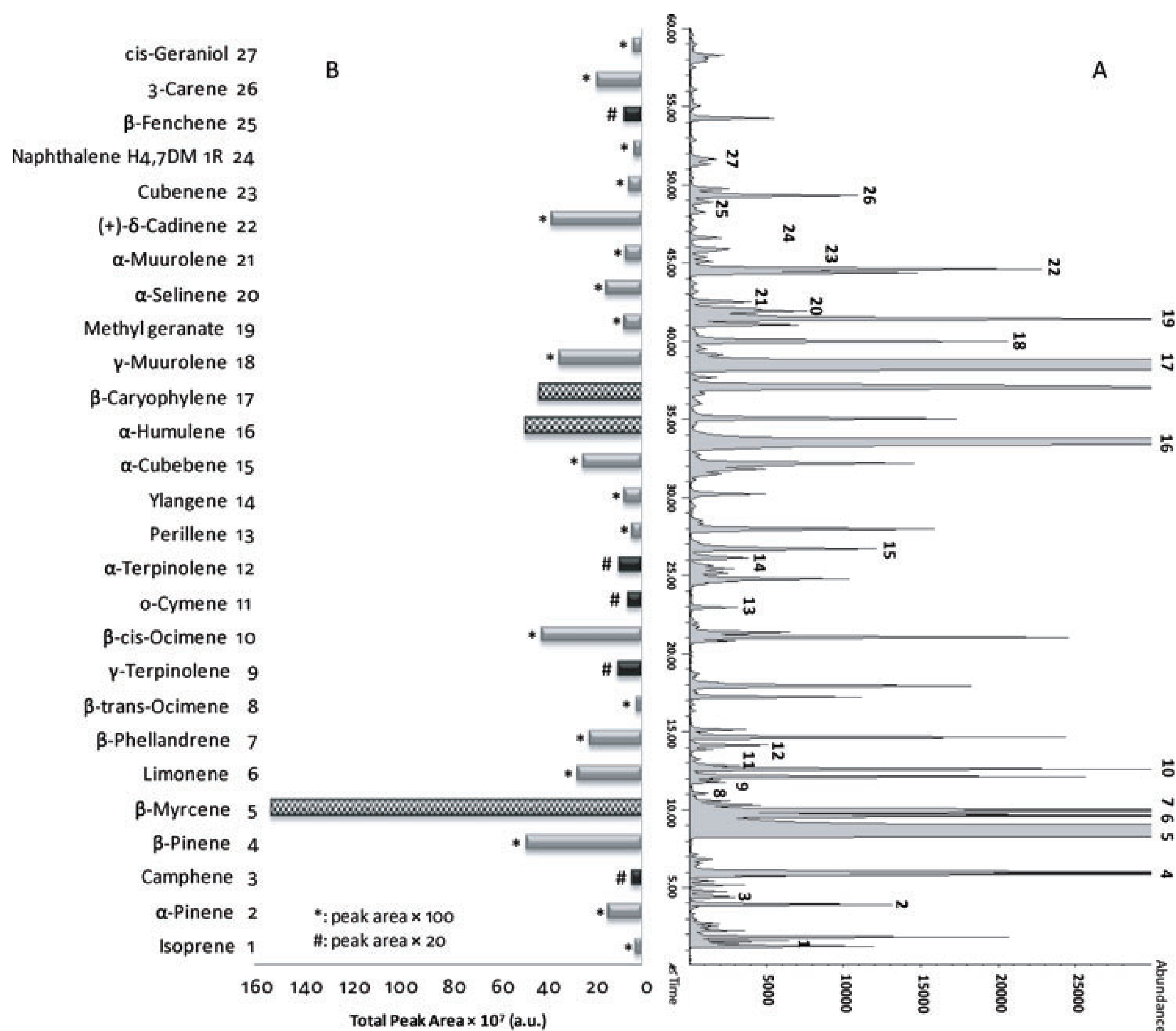

Figure 4. (A) A typical GC-qMS chromatogram of volatile fraction of the *Saaz* hop-essential oil isolated by HS-SPME (extraction conditions: DVB/CAR/PDMS fiber at 40°C during 30 min; for GC-qMS conditions see Section 2.5, for details on peaks identities see Table 2); and (B) pattern of the *Saaz* hop-essential oil terpenoid fraction obtained by HS-SPME$_{DVB/CAR/PDMS}$/GC-qMS methodology.

of *Saaz* variety were 50/30 mm DVB/CAR/PDMS fiber at 40°C for 30 min.

3.1.3 Determination of terpenoid metabolites in hop-essential oil derived from *Saaz* variety by HS-SPME$_{DVB/CAR/PDMS}$/GC-qMS

The optimized HS-SPME/GC-qMS methodological conditions were used for profiling the terpenoid metabolomics pattern of the hop-essential oil from *Saaz* variety. A characteristic GC-qMS profile of *Saaz* hop-essential oil obtained with a DVB/CAR/PDMS fiber using the experimental optimized conditions is shown in Fig. 4A.

A total of 27 terpenoid metabolites (Table 2 and Fig. 4B) were identified in the HS of the essential oil from the *Saaz* hop variety. Among these, 60.7% were monoterpene hydrocarbons, 37.7% were sesquiterpenes hydrocarbons, and it also contained 1.52% oxygenated monoterpenes and 0.04% hemiterpenes. Table 2 shows the identified terpenoid metabolites, their RI values listed in order of elution on a BP-20 capillary column, and the relative composition. The chemical structures of the terpenoid metabolites in the essential oil of hop from *Saaz* variety are summarized in Fig. 5.

The major constituents in the hop-essential oil from *Saaz* variety were the monoterpene (**5**) β-myrcene (53.0 ± 1.1% of the total volatile fraction), and the cyclic sesquiterpenes (**16**) α-humulene (16.6 ± 0.8%) and (**17**) β-caryophyllene (14.7 ± 0.4%), which together account for 84.3 ± 1.5% of the volatile essential oil. Metabolites found at low content include (**4**) β-pinene (1.8%), (**19**) methyl geranate (1.4%), (**10**) β-*cis-o*-cymene (0.7%), (**22**) (+)-δ-cadinene (0.7%), and (**6**) limonene (0.5%). The results are according to Nance and Setzer [52], who reported that the main constituents of hop-essential oil derived from *Saaz* variety are β-myrcene, α-humulene, and β-caryophyllene.

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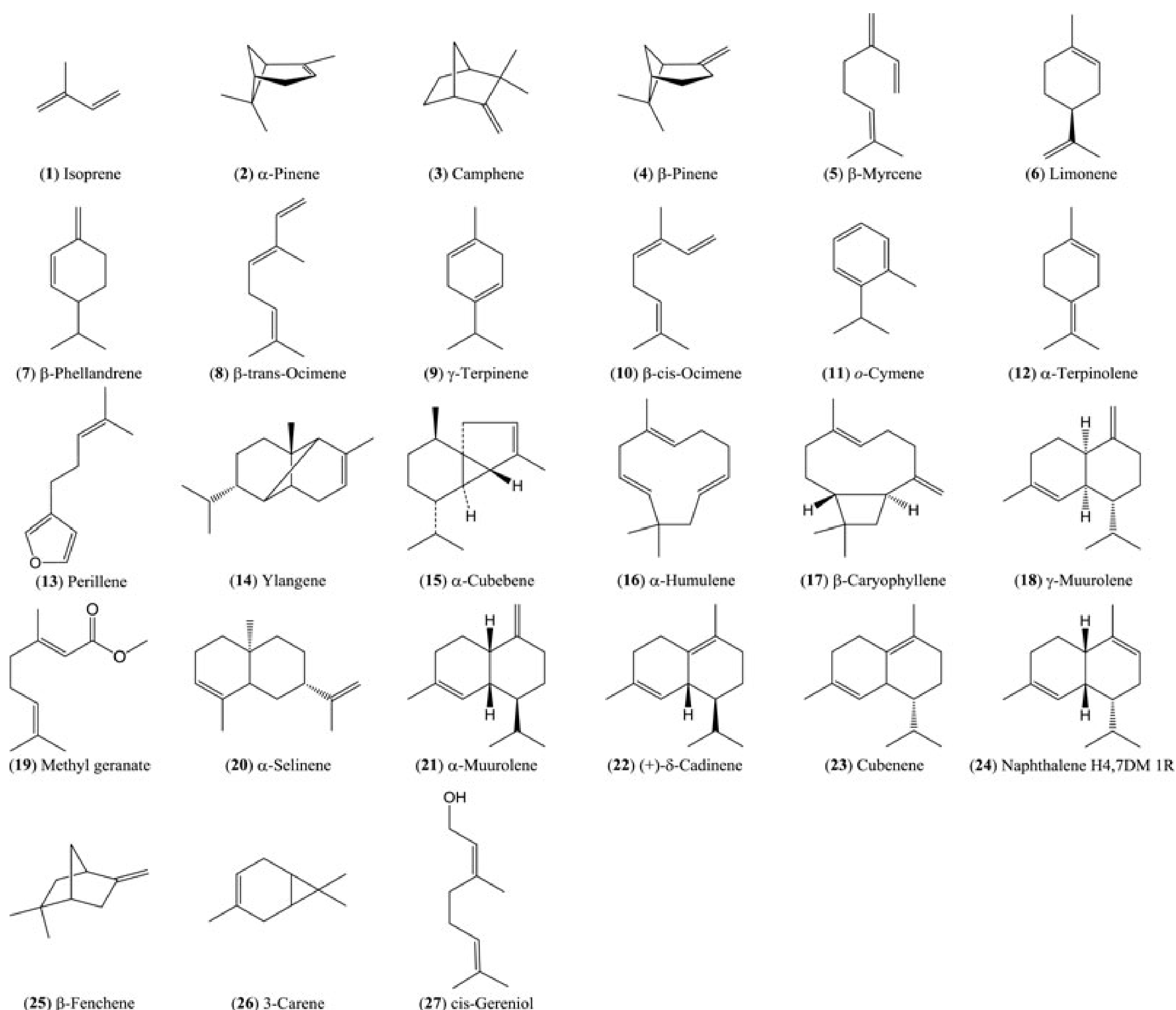

Figure 5. Chemical structures of terpenoid metabolites determined in hop-essential oil from *Saaz* variety.

These compounds are interesting from the standpoint of pharmacological, because of their antimicrobial activity [55] as well as to their anti-inflammatory effects [56]. On the other hand, limonene (**6**) and β-pinene (**4**) have been reported as antitumor [57] and antimicrobial [55] agents, respectively. Biological activities and odor description of some plant secondary metabolites found in hop-essential oil derived from *Saaz* variety are summarized in Table 3.

The odor threshold of β-myrcene (**5**) in water has been determined to range between 13 and 36 ppb [69], and so is expected to exert a large impact on the odor profile of the essential oils. This has been supported in studies using GC-O [3]. It has odor descriptors of resinous, herbaceous, balsamic, and geranium-like [3]. β-Myrcene (**5**) and essential oils containing this monoterpene have been widely used as scenting agents in cosmetics, soaps, detergents, and as flavoring additives in food and beverages. Lorenzetti et al. [70] reported that β-myrcene is a peripheral analgesic substance and the active ingredient in lemongrass (*Cymbopogon citratus*) tea. This potion is widely used in folk medicine to treat gastrointenstinal disturbances and as a sedative and antipyretic [3]. Farnesene was found to be present in some cultivars but not in others. It was not found in the oils of *Saaz* hops. Dehydration of α-terpineol gives limonene (**6**), the major hydrocarbon of citrus oils but also present in hop-essential oil. Limonene (**6**) is probably the precursor of the bicyclic monoterpenes such as α- (**2**) and β-pinene (**4**) and camphene (**3**). Limonene (**6**) can also disproportionate into *o*-cymene (**11**) [3].

4 Concluding remarks

A HS-SPME$_{DVB/CAR/PDMS}$/GC-qMS methodology was developed that allowed to profiling the terpenoid metabolomic pattern of hop-essential oil from *Saaz* variety.

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Table 3. Some selected examples of the biological activity and odor description of terpenic metabolites determined in essential oil from *Saaz* variety [53, 58–68]

Terpene class	Metabolite	Odor description	Biological activity	References
Hemiterpene	Isoprene	Penetrating petroleum-like odor	Thermotolerance. Tolerance of ozone and other reactive oxygen species. "Safety valve" to get rid of unwanted metabolites.	[58]
Monoterpene	α-Pinene	Terpenic, harsh-pine-like, weak herbal	Repels the spruce beetle *Dendroctonus rufipennis* at high concentrations, but intermediate concentrations elicit entry and gallery construction. Elicits olfactory receptor neurons of the weevil *Pissodes notatus*. Enhances attraction by *Thanasimus dubius*, *Platysoma cylindrica*, and *Corticeus parallelus* to the pheromones of their *Ips prey*.	[58]
Monoterpene	Camphene	Camphoraceous-oily	Have been reported to possess biological activity against Gram-positive and Gram-negative bacteria.	[59]
Monoterpene	β-Pinene	Resinous-piney	High/medium antimicrobial effects against both Gram-positive bacteria *Staphylococcus aureus*, *Enterococcus faecalis*, and a high activity against the Gram-negative bacteria *Escherichia coli* and *Proteus vulgaris* as well as a medium one against *Candida albicans*.	[55]
Monoterpene	β-Myrcene	Resinous, herbaceous, balsamic, geranium-like1	High antimicrobial activities only against *Escherichia coli* and *Proteus vulgaris*. Exhibit good repellent activity.	[55]
Monoterpene	Limonene	Mildly citrus, free from, camphoraceous	Lotus japonicus plants infested with two-spotted spider mites (*Tetranychus urticae*). Selection of the oviposition site by predatory hoverflies relies on the perception of a volatile blend composed of prey pheromone and typical plant green leaf volatiles. Elicits olfactory receptor neurons of the weevil *Pissodes notatus*. Involved in the selective herbivory on the conifer *Pinus caribaea* by the leafcutting ant *Atta laevigata*. Limonene has been described in the literature as chemopreventive and therapeutic agent against many tumor cells.	[58]
Monoterpene	β-Phellandrene	Herbaceous, minty background	Have been reported due to effect in inhibition the pathogen *Botrytis cinerea*.	[58]
Monoterpene	β-*trans*-Ocimene	Fresh-terpenic, weak citrus-herbal	Exposure of *Arabidopsis thaliana* to the monoterpene causes increased abundance of several gene transcripts and increased plant resistance against the pathogen *Botrytis cinerea*. Genes of the octadecanoid pathway and genes known to respond to octadecanoids are among the most prevalent within the stress-gene category upregulated in Arabidopsis. The β-ocimene synthase is induced in *Lotus japonicus* plants infested with two-spotted spider mites (*Tetranychus urticae*).	[58]
Monoterpene	α-Terpinene	Herbaceous, citrus	Have been reported to possess a potent repellent activity.	[60]
Monoterpene	o-Cymene	n.i.[a)]	Reveal good antibacterial activity and strong antifungal properties.	[61]
Monoterpene	α-Terpinolene	Pleasant, sweet-piney, somewhat turpentine	Exhibit insect repellent activity. Present a higher relationship with the anticancer activity.	[62]

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Table 3. Continued

Terpene class	Metabolite	Odor description	Biological activity	References
Sesquiterpene	Ylangene	Fruity	Ylangene has been identified as kairomone attractants for male Medfly.	[63]
Sesquiterpene	α-Cubebene	Mild waxy, woody	α-Cubebene has been identified as kairomone attractants for elm bark beetles (Curculionidae: Scolytinae: *Scolytus* spp.).	[64]
Sesquiterpene	α-Humulene	Soft-woody, reminding to fresh earth, balsamic	Produced in high amounts in response to simultaneous herbivory by the piercing–sucking insect western flower thrips *Frankliniella occidentalis* and the chewing herbivore *Heliothis virescens*. Produced by a recombinant insect-induced gene (*AlCarS*) with high sequence similarity to the florally expressed (E)-β-caryophyllene synthase. Medium antimicrobial effects against the Gram-positive bacteria *Enterococcus faecalis* and Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* sp. Reveal an important anti-inflammatory property.	[55, 58]
Sesquiterpene	β-Caryophyllene	Woody, spicy	Elicits electroantennogram responses. Involved in insect host location. Involved in the selective herbivory on the conifer *Pinus caribaea* by the leafcutting ant *Atta laevigata*. Below ground signal emitted by insect damaged maize roots. Induced by a plant pathogen and perceived by its vector insect, the phloem-feeding psyllid *Cacopsylla picta*. Biotransformed by plant-hosted bacteria. Released by Arabidopsis upon insect feeding. Strong repellent activity against *A. aegypti*. High antimicrobial activities against the Gram-negative-bacteria *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Salmonella* sp. as well as medium effects against the Gram-positive bacteria *Enterococcus faecalis* and the Gram-negative bacteria *Escherichia coli*. Exhibit an important anti-inflammatory property.	[55, 58]
Sesquiterpene	γ-Muurolene	Oily, Herbaceous	Exhibit antifungal and antibacterial activity.	[53]
Oxy-monoterpene	Methyl geranate	Flower, green, fruit	Methyl geranate has been identified as pheromone produced by *Chlorochroa sayi* males.	[65]
Sesquiterpene	α-Selinene	Weak spicy, balsamic, mild	Exhibit antifungal and antibacterial activity.	[53]
Sesquiterpene	α-Muurolene	Woody	Possess antifungal activity against *Cladosporium cucumerinum*.	[66]
Sesquiterpene	(+)-δ-Cadinene	Herbaceous	High antimicrobial activities against *Streptococcus pneumoniae*. (+)-δ-cadinene is an early enzymatic intermediate in the biosynthesis of the sesquiterpenoid phytoalexins by upland cotton	[67]
Sesquiterpene	Cubenene	Weak woody, herbal, spicy	α-Cubenene has been identified as kairomone attractants for elm bark beetles (Curculionidae: Scolytinae: *Scolytus* spp.).	[68]
Monoterpene	3-Carene	Citrus fruit, orange peel	Exhibit repellent activity against *Anopheles gambiae*.	[62]
Oxy-monoterpene	*cis*-Geraniol	Floral-rose, geranium	Reveal antibacterial activity at a broad spectrum. Exhibit insect repellent activity.	[62]

a) No information.

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The importance of terpenoids is not limited to their aromatic properties; they are also associated to insect repellent activity and to desirable properties in the human health.

Concerning the application of the developed methodology to the *Saaz* hop-essential oil, it was possible to identify in their HS 27 terpenoids, which include 13 monoterpenes, ten sequiterpenes, three oxygenated monoterpenes, and one hemiterpene. According to GC-qMS analysis, the terpenoid fraction of the *Saaz* hop-essential oil is dominated by the monoterpene β-myrcene, and the cyclic sesquiterpenes α-humulene, and β-caryophyllene, which together account for 84% of the volatile hop-essential oil. Considering the wide range of biological and pharmacological activities associated to terpenoid metabolites, and taking into account the highest content of these metabolites in hop-essential oil derived from *Saaz* variety, we can estimate that this matrix can be used as a powerful and valuable natural biosource of terpenoid metabolites. In brief, our findings suggested that the essential oil derived from hop *Saaz* variety and its effective constituents can be explored as a powerful biological source for newer, more selective, biodegradable and naturally produced antimicrobial and antioxidant compounds, as an environmentally friendly alternative to synthetic chemicals to control some bacterial strains, fungs, and insects.

Future works will include studies on biological activity of the hop-essential oil through the evaluation of their antibacterial, antifungal, and antioxidant activity.

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The authors have declared no conflict of interest.

5 References

[1] Karuppusamy, S., *J. Med. Plant Res.* 2009, *3*, 1222–1239.

[2] Wang, G., Tang, W., Bidigare, R. R., in: Zhang, L., Demain, A. L. (Eds.), *Natural Products*, Humana Press, New Jersey 2005, pp. 197–227.

[3] Preedy, V., *Beer in Health and Disease Prevention*, Academic Press, London 2009.

[4] Demain, A., Fang, A., in: Fiechter, A. (Ed.), *History of Modern Biotechnology I*, Springer, Berlin/Heidelberg 2000, pp. 1–39.

[5] Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., *Food Chem. Toxicol.* 2008, *46*, 446–475.

[6] Daayf, F., Lattanzio, V., *Recent Advances in Polyphenol Research*, Blackwell Publishing, Oxford 2008.

[7] Zwenger, S., Basu, C., *Biotechnol. Mol. Biol. Rev.* 2008, *3*, 1–7.

[8] Aharoni, A., Jongsma, M. A., Bouwmeester, H. J., *Trends Plant Sci.* 2005, *10*, 594–602.

[9] Jaenson, T. G. T., Pålsson, K., Borg-Karlson, A.-K., *J. Med. Entomol.* 2006, *43*, 113–119.

[10] Gillij, Y. G., Gleiser, R. M., Zygadlo, J. A., *Bioresour. Technol.* 2008, *99*, 2507–2515.

[11] Kiran, S. R., Devi, P. S., *Parasitol. Res.* 2007, *101*, 413–418.

[12] Burke, Y., Stark, M., Roach, S., Sen, S., Crowell, P., *Lipids* 1997, *32*, 151–156.

[13] Joo, J. H., Jetten, A. M., *Cancer Lett.* 2010, *287*, 123–135.

[14] Khan, R., Sultana, S., *Chem. Biol. Interact.* 2011, *192*, 193–200.

[15] Unnanuntana, A., Bonsignore, L., Shirtliff, M. E., Greenfield, E. M., *J. Bone Joint Surg. Am.* 2009, *91*, 2683–2692.

[16] Khalil, A. A., Davies, B., Castagnoli Jr., N., *Bioorg. Med. Chem.* 2006, *14*, 3392–3398.

[17] Ikeda, T., Yokomizo, K., Okawa, M., Tsuchihashi, R., Kinjo, J., Nohara, T., Uyeda, M., *Biol. Pharm. Bull.* 2005, *28*, 1779–1781.

[18] Jeppesen, P. B., Gregersen, S., Poulsen, C. R., Hermansen, K., *Metabolism* 2000, *49*, 208–214.

[19] Peana, A. T., D'Aquila, P. S., Chessa, M. L., Moretti, M. D. L., Serra, G., Pippia, P., *Eur. J. Pharmacol.* 2003, *460*, 37–41.

[20] Paduch, R., Kandefer-Szerszeń, M., Trytek, M., Fiedurek, J., *Arch. Immunol. Ther. Exp.* 2007, *55*, 315–327.

[21] Adam, R. P., *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, Allured Publishing Corporation, Illinois 2007.

[22] Zygadlo, J. A., Juliani, H. R., in: Majundar, D. K., Govil, J. N., Singh, V. K., Shailaja, M. S., Gangal, S. V. (Eds.), *Phytochemistry and Pharmacology*, Studium Press LLC, Texas 2003, pp. 273–281.

[23] Hopsteiner, *Hop Varieties*, S.S. Steiner, Inc., New York 2011.

[24] Hopunion, L., *Hop Variety Handbook*, Hopunion LLC, Yakima, Washington, DC 2011.

[25] Wainwright, T., *Basic Brewing Science: An Introduction to the Scientific Basis of Malting and Brewing for People Employed in the Malting, Brewing, or Associated Industries*, Magic Print Limited, South Africa 1998.

[26] Bozin, B., Mimica-Dukic, N., Simin, N., Anackov, G., *J. Agric. Food Chem.* 2006, *54*, 1822–1828.

[27] Nickerson, G. B., Van Engel, E. L., *J. Am. Soc. Brew. Chem.* 1992, *50*, 77–81.

[28] Steinhaus, M., Schieberle, P., *J. Agric. Food Chem.* 2000, *48*, 1776–1783.

[29] del Valle, J. M., Rivera, O., Teuber, O., Palma, M. T., *J. Sci. Food Agric.* 2003, *83*, 1349–1356.

[30] Yang, H., Li, X., Zhen, J., Chen, F., *J. Sep. Sci.* 2009, *32*, 3152–3156.

[31] Qu, B., Cheng, Y., *J. Sep. Sci.* 2007, *30*, 1284–1291.

[32] Caldeira, M., Rodrigues, F., Perestrelo, R., Marques, J. C., Câmara, J. S., *Talanta* 2007, *74*, 78–90.

[33] Câmara, J. S., Marques, J. C., Perestrelo, R. M., Rodrigues, F., Oliveira, L., Andrade, P., Caldeira, M., *J. Chromatogr. A* 2007, *1150*, 198–207.

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[34] Duan, B., Huang, L., Chen, S., *J. Sep. Sci.* 2012, *35*, 513–518.

[35] t'Kindt, R., Alaerts, G., Heyden, Y. V., Deforce, D., Bocxlaer, J. V., *J. Sep. Sci.* 2007, *30*, 2002–2011.

[36] Mendes, B., Gonçalves, J., Câmara, J. S., *Talanta* 2012, *88*, 79–94.

[37] Ivanova, s. A., Shikov, A. N., Makarov, V. G., *J. Sep. Sci.* 2006, *29*, 2245–2250.

[38] Lord, H. L., Pawliszyn, J., *Anal. Chem.* 1997, *69*, 3899–3906.

[39] Arthur, C. L., Killam, L. M., Buchholz, K. D., Pawliszyn, J., Berg, J. R., *Anal. Chem.* 1992, *64*, 1960–1966.

[40] Pereira, J., Pereira, J., Câmara, J. S., *Talanta* 2011, *83*, 899–906.

[41] Gonzalez, A. G., Zavala, L. C., Moreno, A. P., San Juan, E. R., Ferrara, J. G., Espinosa, L. R., Jimenez, G. M., *Bioanalysis* 2011, *3*, 439–448.

[42] Ferreira, L., Perestrelo, R., Caldeira, M., Câmara, J. S., *J. Sep. Sci.* 2009, *32*, 1875–1888.

[43] Ferreira, L., Perestrelo, R., Câmara, J. S., *Talanta* 2009, *77*, 1087–1096.

[44] Pontes, M., Marques, J. C., Câmara, J. S., *Microchem. J.* 2009, *93*, 1–11.

[45] Ouyang, G., Pawliszyn, J., *TrAC, Trends Anal. Chem.* 2006, *25*, 692–703.

[46] Alizadeh, N., Mohammadi, A., Tabrizchi, M., *J. Chromatogr. A* 2008, *1183*, 21–28.

[47] Musteata, F. M., Pawliszyn, J., *TrAC, Trends Anal. Chem.* 2007, *26*, 36–45.

[48] Silva, C. L., Passos, M., Camara, J. S., *Br. J. Cancer* 2011, *105*, 1894–1904.

[49] Silva, C. L., Passos, M., Câmara, J. S., *Talanta* 2012, *89*, 360–368.

[50] van Den Dool, H., Dec Kratz, P., *J. Chromatogr. A* 1963, *11*, 463–471.

[51] Campo, L., Mercadante, R., Rossella, F., Fustinoni, S., *Anal. Chim. Acta* 2009, *631*, 196–205.

[52] Nance, M. R., Setzer, W. N., *J. Brew. Distilling* 2011, *2*, 16–22.

[53] Koroch, A. R., Juliani, H. R., Zygadlo, J. A., in: Berger, R. G. (Ed.), *Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability*, Springer, Berlin 2007, pp. 87–116.

[54] Lord, H., Pawliszyn, J., *J. Chromatogr. A* 2000, *902*, 17–63.

[55] Jirovetz, L., Bail, S., Buchbauer, G., Denkova, Z., Slavechev, A., Stoyanova, A., Schmidt, E., *Sci. Pharm.* 2006, *74*, 189–201.

[56] Fernandes, E. S., Passos, G. F., Medeiros, R., da Cunha, F. M., Ferreira, J., Campos, M. M., Pianowski, L. F., Calixto, J. B., *Eur. J. Pharmacol.* 2007, *569*, 228–236.

[57] Rabi, T., Bishayee, A., *Breast Cancer Res. Treat.* 2009, *115*, 223–239.

[58] Maffei, M. E., *S. Afr. J. Bot.* 2010, *76*, 612–631.

[59] Yamaguchi, M. U., da Silva, A. P. B., Ueda-Nakamura, T., Filho, B. P. D., da Silva, C. C., Nakaruma, C. V., *Molecules* 2009, *14*, 1796–1807.

[60] Choi, W. S., Park, B. S., Ku, S. K., Lee, S. E., *J. Am. Mosq. Control Assoc.* 2002, *18*, 348–351.

[61] Gundidza, M., Gweru, N., Magwa, M. L., Ramalivhana, N. J., Humphrey, G., Samie, A. V. M., *Afr. J. Biotechnol.* 2009, *8*, 721–724.

[62] Nerio, L. S., Olivero-Verbel, J., Stashenko, E., *Bioresour. Technol.* 2010, *101*, 372–378.

[63] Metcalf, R. L., Metcalf, E. R., *Plant Kairomones in Insect Ecology and Control*, Chapman & Hall, Inc., London 1992.

[64] Gullan, P. J., Cranston, P. S., *The Insects: An Outline of Entomology*, Wiley-Blackwell, Oxford 2010.

[65] Millar, J. G., in: Schulz, S. (Ed.), *The Chemistry of Pheromones and Other Semiochemicals II*, Springer Verlag, Berlin, Heidelberg 2005, pp. 37–84.

[66] Ali, N. A. A., Wurster, M., Arnold, N., Lindequist, U., Wessjohan, L., *Rec. Nat. Prod.* 2008, *2:3*, 70–75.

[67] Pérez-Lopéz, A., Cirio, A. T., Rivas-Galindo, V. M., Aranda, R. S., de Torres, N. W., *J. Essent. Oil Res.* 2011, *23*, 25–28.

[68] Gullan, P. J., Cranston, P., *The Insects: An Outline of Entomology*, Wiley-Blackwell, Oxford 2004.

[69] Masanetz, C., Grosch, W., *Flavour Fragrance J.* 1998, *13*, 115–124.

[70] Lorenzetti, B. B., Souza, G. r. E. P., Sarti, S. I. J., Santos Filho, D., Ferreira, S. r. H., *J. Ethnopharmacol.* 1991, *34*, 43–48.

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