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Talanta 74 (2007) 91–103

talanta

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Screening of volatile composition from Portuguese multifloral honeys using headspace solid-phase microextraction-gas chromatography–quadrupole mass spectrometry

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Received 15 March 2007; received in revised form 14 May 2007; accepted 18 May 2007
Available online 26 May 2007

Abstract

The volatile composition from four types of multifloral Portuguese (produced in Madeira Island) honeys was investigated by a suitable analytical procedure based on dynamic headspace solid-phase microextraction (HS-SPME) followed by thermal desorption gas chromatography–quadrupole mass spectrometry detection (GC–qMS). The performance of five commercially available SPME fibres: 100 μm polydimethylsiloxane, PDMS; 85 μm polyacrylate, PA; 50/30 μm divinylbenzene/carboxen on polydimethylsiloxane, DVB/CAR/PDMS (StableFlex); 75 μm carboxen/polydimethylsiloxane, CAR/PDMS, and 65 μm carbowax/divinylbenzene, CW/DVB; were evaluated and compared. The highest amounts of extract, in terms of the maximum signal obtained for the total volatile composition, were obtained with a DVB/CAR/PDMS coating fibre at 60 °C during an extraction time of 40 min with a constant stirring at 750 rpm, after saturating the sample with NaCl (30%). Using this methodology more than one hundred volatile compounds, belonging to different biosynthetic pathways were identified, including monoterpenols, C_{13}-norisoprenoids, sesquiterpenes, higher alcohols, ethyl esters and fatty acids. The main components of the HS-SPME samples of honey were in average ethanol, hotrienol, benzeneacetaldehyde, furfural, *trans*-linalool oxide and 1,3-dihydroxy-2-propanone.
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Keywords: Honey; Volatile compounds; Solid-phase microextraction; GC–qMS

1. Introduction

Honey is a natural product produced by *Apis mellifera* bees from the nectar of plants and has for long been an excellent nutritional option for many generations due to its health benefits (one of the traditional sources for treatment of flue and common cold in the region) [1], has been reported to be effective in gastrointestinal disorders, in healing of wounds and burns, as an anti-microbial agent [2]. The healing effect of honey is due to the enzyme glucose oxidase, this enzyme is virtually inactive in full-density honey but becomes active in diluted honey producing hydrogen peroxide and gluconic acid from glucose. In addition, many natural antibacterial compounds have been identified from different types of honey [2]. Honey, as a source of antioxidants has been proven to be effective against deterioative oxidation reaction in food [3]. The antibacterial activity of honey is attributed both to physical factors, acidity and osmolarity and chemical factors, hydrogen peroxide, volatiles, beeswax, nectar, pollen and propolis [2,3].

Honey includes over 400 different chemical compounds, more than 95% mainly formed by sugars and water, whereas proteins, vitamins (mainly vitamin B6, thiamin, niacin, riboflavin, and pantothenic acid), essential minerals (including calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), pigments, flavours, free amino acids and volatile compounds constitute minor components [4]. The sugars present in honey are mainly fructose (about 38.5%) and glucose (about 31.0%). The remaining carbohydrates include maltose, sucrose, and other complex carbohydrates.

The chemical composition of honey is highly dependent to the nectar source and the botanical origin of the nectar foraged by bees [1]. Aroma compounds are present in honey at very low concentrations as complex mixtures of volatile components of different chemical families belonging, in general, to

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0039-9140/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2007.05.037

monoterpenes, C_{13}-norisoprenoids, sesquiterpenoids, benzene derivatives [5] and in lowest content, higher alcohols, esters, fatty acids, ketones, terpenes and aldehydes. Some of these substances are present in honey collected by bees, and have been described as characteristics of the floral source (could be related to plant characteristics), and other compounds, like some alcohols, branched aldehydes, and furfural derivatives, may be related to the microbial purity of processing and storage conditions of honey [6]. The quantitative analysis of volatile compounds present in such samples is extremely demanding due: (1) to complex chemical composition of the volatile fraction and (2) the fact of individual volatile compounds can be present in a wide range of concentration. Honey volatile fractions have been used as quality markers for the authenticity of the floral origin [7,8]. It also prevents overpayment and helps to identify frauds [7].

Traditional analytical methods employing organic solvents such as liquid–liquid extraction [9], simultaneous distillation–extraction (SDE) [10], supercritical fluid extraction (SFE) [11], solid-phase extraction (SPE) [12] and ultrasound extraction [13] were commonly used. These are hazardous since requires large amounts of toxic and expensive solvents, are labour-intensive and time-consuming and requires the preconcentration of the extract. Each procedure of the sample preparation is subject to inconveniences, but offers specific advantages under determined circumstances. Nowadays, alternative to these classical methods that may overcome their disadvantages, more easier and selective, are used such as solid-phase microextraction (SPME), developed by Pawliszyn and coworker [14,15] in the early 1990s and more recently stir bar sorptive extraction (SBSE) developed in the late 1990s by Baltussen et al. [16]. This technique uses a TwisterTM, a glass stir bar onto which is bonded a sorptive phase, often polydimethylsiloxane (PDMS), in quantities far in excess of those found on SPME fibres [17]. SPME is an equilibrium technique that requires a previous optimisation of the extraction parameters that can affect extraction efficiencies, in order to obtain high recoveries of volatiles. SPME sampling can be performed in three basic modes: direct extraction (the analytes were transported directly from matrix to the extracting phase), headspace extraction (the analytes are extracted from the gas phase equilibrated with the sample) and extraction with membrane protection (the fibre is separated from the sample with a selective membrane). The selection of the sampling mode is dependent of the nature of the compounds to be analysed and the sample type. Bearing in mind that one of the goals of this study was to screen volatile compounds from honeys, the headspace sampling mode was the most appropriate. The headspace SPME process protects the fibre from adverse effects caused by non-volatile compounds present in the sample matrix namely sugars, and allows modifications, as for example: pH, with no effect in the fibre. Moreover, the equilibration times for volatile compounds are shorter for headspace SPME extraction than for direct extraction under the same conditions.

Since the first SPME fibres becomes commercially available, it has been more and more used and the fields of application have been continuously growing, including a wide range of food analysis, namely the volatile composition of wines [18–22], beers [23], whiskeys [24–26], several kinds of fruits [27–31] and honeys [32–36], with nowadays about 3000 research papers published. The technique gained growing acceptance and increasing use in routine laboratories and industrial applications. This method shows clear advantages compared with traditional techniques, eliminates the use of (toxic) organic solvents, allows the quantification of a large number of molecules, no or little manipulation/preparation of samples, substantially shortness the time of analysis and moreover are simple and faster techniques, and covers a wide range of sampling techniques, including field, *in situ* and air sampling. Generally accepted disadvantages are relatively lot-to-lot variations, sensitivity against organic solvents and the limited range of stationary phases which are commercially available.

In this study, headspace SPME combined with GC–qMS, was developed and applied to evaluating the volatile composition profile of different multifloral honey samples (H1–H4). A preliminary screening of fibre of various polarities was carried out in order to select the best coating for the matrix. Comparison between the performance of the five sorbent materials is given. To confirm the applicability of the SPME, comparative study on the characteristic GC–qMS volatile honey profiles were performed. The possibility of differentiation from the investigated honeys was evaluated.

2. Experimental

2.1. Chemicals and materials

All reagents used were analytical quality and all solvents were HPLC grade. Sodium chloride (99.5%) used to obtain the adequate ionic strength (decrease the solubility of the aroma molecules which then partition more readily into the headspace improving the adsorption of analytes in SPME analysis), was supplied by Merck (Darmstadt, Germany). Water was purified through a Milli-Q purification system (Millipore). The C_8–C_{20} *n*-alkanes series, and the chemical standard used as internal standard, 3-octanol, were supplied by Sigma–Aldrich (Spain).

The SPME fibres tested and compared in this work, polydimethylsiloxane layer (PDMS, 100 μm), recommended for nonpolar volatiles; polyacrylate (PA, 85 μm) with high selectivity for polar semivolatile compounds; divinylbenzene–carboxen–polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm) on a 1 cm StableFlex fibre, recommended for flavours (volatiles and semivolatiles); carboxen–polydimethylsiloxane (CAR/PDMS, 75 μm); carbowax–divinylbenzene (CW/DVB, 65 μm) and the SPME holder for manual sampling, were obtained from Supelco (Bellefonte, PA, USA). The coating of all fibres was 1 cm long. The fibres were conditioned prior to use according to the manufacturer's instructions by inserting them into the GC injector port. Before the first daily analysis the fibres were conditioned for 20 min. A blank test was performed to check possible carry-over.

2.2. Honey samples

This study was carried out on 16 multifloral honeys, H1–H4 (four samples from each honey) from four different regions of Madeira Island (Portugal) where predominates the wild flora, eucalyptus, hissed and rosemary. All samples were obtained from local stores and were processed using the traditional procedures. None of these samples underwent any treatment that could alter their composition.

All samples were contained in glass bottles and stored at 4 °C until analysis. All analysis, were carried out four times.

2.3. SPME methodology

In order to select the best fibre for honey volatile composition, five SPME coatings of different polarity and extraction mechanisms were tested. The methodology developed and optimised by Câmara et al. [37] for the volatile compounds extraction using manual SPME was used in this study with minor modifications. The H1 honey sample was selected as the matrix for comparison of the performance of the fibres. The fibre that presented the most complete profile of H1 honey volatile compounds was chosen for the study. The extraction was carried out at 60 °C (controlled temperature) and each measurement was repeated four times.

For each extraction 15 g of sample was diluted 1:1 with deionised water (Milli-Q). The dilution decrease the density of the matrix making easy the evaporation of analytes of the interest otherwise retained for sugars. For headspace sampling, 30 mL of the sample was immediately placed in a 60 mL ambar glass vial (volume ratio headspace/solution was 1:1) hermetically sealed and spiked with 3-octanol (250 μL at 422 mg L^{-1}) and 4-methyl-2-pentanol (125 μL at 422 mg L^{-1}) (Sigma–Aldrich) used as internal standards, and equilibrated during 40 min in a thermostatic bath on a stirrer. The extractions were carried out without adjust of pH. The ionic strength was increased using NaCl (30%, w/v) to improve the extraction efficiency by decreasing the solubility of hydrophilic compounds in the aqueous phase. The SPME fibre was then exposed to the headspace of the sample and kept for 40 min at 60 °C. As stirring usually improves the extraction, because the static layer resistant to mass transfer is destroyed (facilitate mass transport between the bulk of the aqueous sample and the fibre), all the experiments were performed under constant stirring velocity (750 rpm).

After sampling, the SPME fibre was withdrawn into the needle, removed from the vial and inserted into the hot injector port (260 °C) of the GC–qMS system for 6 min where the extracted analytes were thermally desorbed and transferred directly to the analytical column.

2.4. Gas chromatography–quadrupole mass spectrometry detection (GC–qMS)

The volatile compounds extracted by dynamic headspace SPME procedure from honey, were tentatively identified using an Agilent 6890N (Palo Alto, CA, USA) gas chromatograph system coupled to an Agilent 5975 quadrupole inert mass selective detector equipped with a 30 m × 0.25 mm i.d., with a 0.25 μm film thickness, BP-20 fused silica capillary column, according to the method described by Câmara et al. [37]. Helium (Helium N60, Air Liquid, Portugal) was used as the carrier gas at a flow rate ≈ 1 mL min^{-1} (column-head pressure: 13 psi). An insert of 0.75 mm i.d. was used and the injector temperature was fixed at 260 °C. Splitless injections were used. The temperature used included the following settings: initial temperature of 50 °C was held for 1 min and then increased in three steps: 50–100 °C, at 2.5 °C min^{-1}; 100–180 °C, at 2 °C min^{-1} and 180–220 °C, at 15 °C min^{-1}. Each step was preceded by a small period at constant temperature for 2, 1 and 10 min, respectively. The manifold, GC–qMS interface and quadrupole temperatures were held at 180, 220 and 180 °C, respectively. The detection was performed by a 5975 mass spectrometer in the electronic impact (EI) mode (ionization energy, 70 eV; source temperature, 180 °C). The electron multiplier was set to the auto tune procedure. The mass acquisition range, made in full scan mode, was 30–300 *m/z*; 1.9 spectra/s.

Compounds were identified by comparing the MS fragmentation pattern with those of the pure standards and mass spectrum of the unknown peaks with those stored in the NIST GC/MS library, retention time of the pure standards obtained under the same conditions, and Kováts retention indices (RI). For the determination of the RI a C_8–C_{20} *n*-alkanes series was used. The relative amounts of individual components are expressed as percent peak areas (RPA, %) relative to the total peak area.

2.5. Statistical analysis

Significant differences among the honey samples were determined by one-way analysis of variance (ANOVA) using a SPSS Program, Version 14.0 (SPSS Inc., 2006). Principal component analysis (PCA) and stepwise linear discriminant analysis (SLDA) were performed using the same SPSS program. These techniques were applied to the normalized total peak areas from different chemical classes.

3. Results and discussion

Sixteen honey samples from different multifloral origins were analysed with the objective to identify and compare their volatile compounds profiles. Differences in the total ion current (TIC) chromatographic profiles were observed when comparing the studied honeys. TIC chromatograms from H1–H4 samples were compared in terms of total areas of the volatile compounds and number of compounds. The identified compounds were organized in different groups according to their chemical structure. This was done for C_{13}-norisoprenoids/monoterpenes, sesquiterpenoids, higher alcohols, fatty acids, ethyl esters, furanic compounds, carbonyl compounds, aliphatic and aromatic hydrocarbons, while compounds of different structures were considered together as the class “miscellaneous”.

From these identification we found that carbonyl compounds, furanic compounds, higher alcohols and C_{13}-

norisoprenoids/monoterpenes, constitute a main part of flavour studied honeys, and they probably play a significant role in their flowery notes. These founds are in good agreement with previous reports [34,35]. As predominant compounds *trans*-linalool oxide, furfural, hotrienol and in minor extent, 1,3-dihydroxy-2-propanone, 5-hydroxymethylfurfural, benzeneacetaldehyde, ethyl decanoate, ethyl dodecanoate, *o*-methoxyacetophenone and 2-ethyl hexanoic acid were found. The two fatty acid ethyl esters may contribute with sweety and fruity notes while benzeneacetaldehyde may contribute with flowery, rose and honey notes.

Furanic compounds such as furfural, 1-(2-furanyl)-ethanone and 5-hydroxymethylfurfural were presented in all samples. Several aliphatic and aromatic hydrocarbons arise from bee wax which has not been completely separated during harvest and processing, were identified.

3.1. Selection of SPME fibre coating

The fibre coating used influences the chemical nature of the extracted analyte that is established by their characteristic polarity and volatility. To evaluate the extraction yields of the honey volatile components by HS-SPME, and taking account of the physico/chemical characteristics of the targets under consideration, we tested five types of fibre (CAR/DVB, CW/PDMS, PDMS, PA, and DVB/CAR/PDMS) among those used most routinely for assaying volatiles. A few key experimental factors which influence the HS-SPME extraction yield, namely time required for the target analytes to reach equilibrium and extraction temperature, were previously evaluated by Câmara et al. [37], and applied in this work. Bearing in mind the obtained results, each fibre was exposed to the headspace at the same temperature (60 °C) during the same extraction time (40 min). For reasons of comparability all tests were carried out with the same honey sample (H1). The comparison of the SPME fibre performance was made in terms of extraction efficiency, number of identifiable compounds in the extract and reproducibility. The chromatographic profiles presented in Fig. 1, obtained for a H1 honey sample by using different coatings in same experimental conditions, shows the different extraction efficiency of the fibres. The results obtained using the five fibres on the same H1 sample, in rigorously reproduced temperature and exposure time conditions, are reported in Table 1 . The qualitative composition of honey volatiles and the number of identified compounds using the five fibres under study is very different. As can be seen in Table 1 with DVB/CAR/PDMS fibre a total of 46 compounds were identified whilst with the PA, CW/DVB, CAR/PDMS and PDMS coatings, were detected only, 32, 30, 35 and 17 compounds, respectively. The semi-polar DVB/CAR/PDMS fibre allowed the best efficiency of extraction for volatile compounds while under the same conditions PDMS fibre had the lowest sorption capacity. The former fibre provided the best sensitivity in terms of total compound peak areas, highest number of detected compounds and high reproducibility, hence this fibre was chosen for evaluation of volatile compounds in honeys (Table 2). PA fibre coating extracted 71.8% of DVB/CAR/PDMS while PDMS coating extracted the lowest amount (about 32.0% of DVB/CAR/PDMS). Similar amounts of volatiles (57.4–60.2% of DVB/CAR/PDMS) were extracted when using either, CW/DVB or CAR/PDMS coatings (Table 2). From Fig. 2, it can be observed that the fibres show different selectivity to different target compounds.

Higher alcohols (HA) have a larger affinity for PA fibre. This coating also present better sensitivity for furanic compounds (FC), carbonyl compounds (CaC) and miscellaneous. DVB/CAR/PDMS coating showed a strong extraction capacity for aliphatic and aromatic hydrocarbons (HSHAr), C_{13}-norisoprenoids/monoterpenes (NT), sesquiterpenoids (S) and ethyl esters (EEs).

Some characteristic honey compounds were isolated by the five fibres, such as toluene, *trans*-linalool oxide, furfural, linalool, hotrienol, benzeneacetaldehyde, 1,3-dihydroxy-2-propanone, 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4one (DDMP) and 5-hydroxymethylfurfural.

3.2. Volatile compounds in honey samples

The proposed HS-SPME method, previously optimised and validated, was applied to determine the content of volatile patterns in four commercial available Portuguese honeys. Each honey was analysed four times using the best sampling conditions described above. A characteristic GC–qMS profile of each honey obtained with DVB/CAR/PDMS using the experimental conditions discussed above is shown in Fig. 3. More than 100 volatile compounds belonging to several chemical classes were positively identified, including monoterpenes, C_{13}-norisoprenoids, sesquiterpenoids, higher alcohols, fatty acids ethyl esters, fatty acids, furanic compounds, carbonyl compounds and aliphatic/aromatic hydrocarbons. Most of the volatile compounds were identified by a library search NIST. In some cases a comparison with authentic compounds was performed. The Kováts retention indices were calculated for each peak and compared with the literature in order to ensure the correct identification of the compounds. Table 3summarises the average ($n = 4$) relative composition determined in the mutifloral studied honeys. The relative composition of every flavour compound was calculated as the percent ratio of the respective peak area relative to the total peak area (RPA, %) and relative to the compound with highest peak area in each honey (RPC, %).

A total of 110 volatile compounds were identified in investigated honey samples. Fifteen of these volatiles: ethanol, toluene, 1-methyl-2-(1-methylethyl)-benzene, *trans*-linalool oxide, acetic acid, furfural, 1-(2-furanyl)-ethanone, linalool, hotrienol, benzeneacetaldehyde, 1-nonanol, 1,3-dihydroxy-2-propanone, nonanoic acid, DDMP and 5-hydroxymethylfurfural, were detected in all samples (Table 3) but the ratio between the single components were different for each honey. Some of these 15 compounds ethanol, toluene, *trans*-linalool oxide, furfural, hotrienol and benzeneacetaldehyde, have been reported as common components of various honeys [32–36].

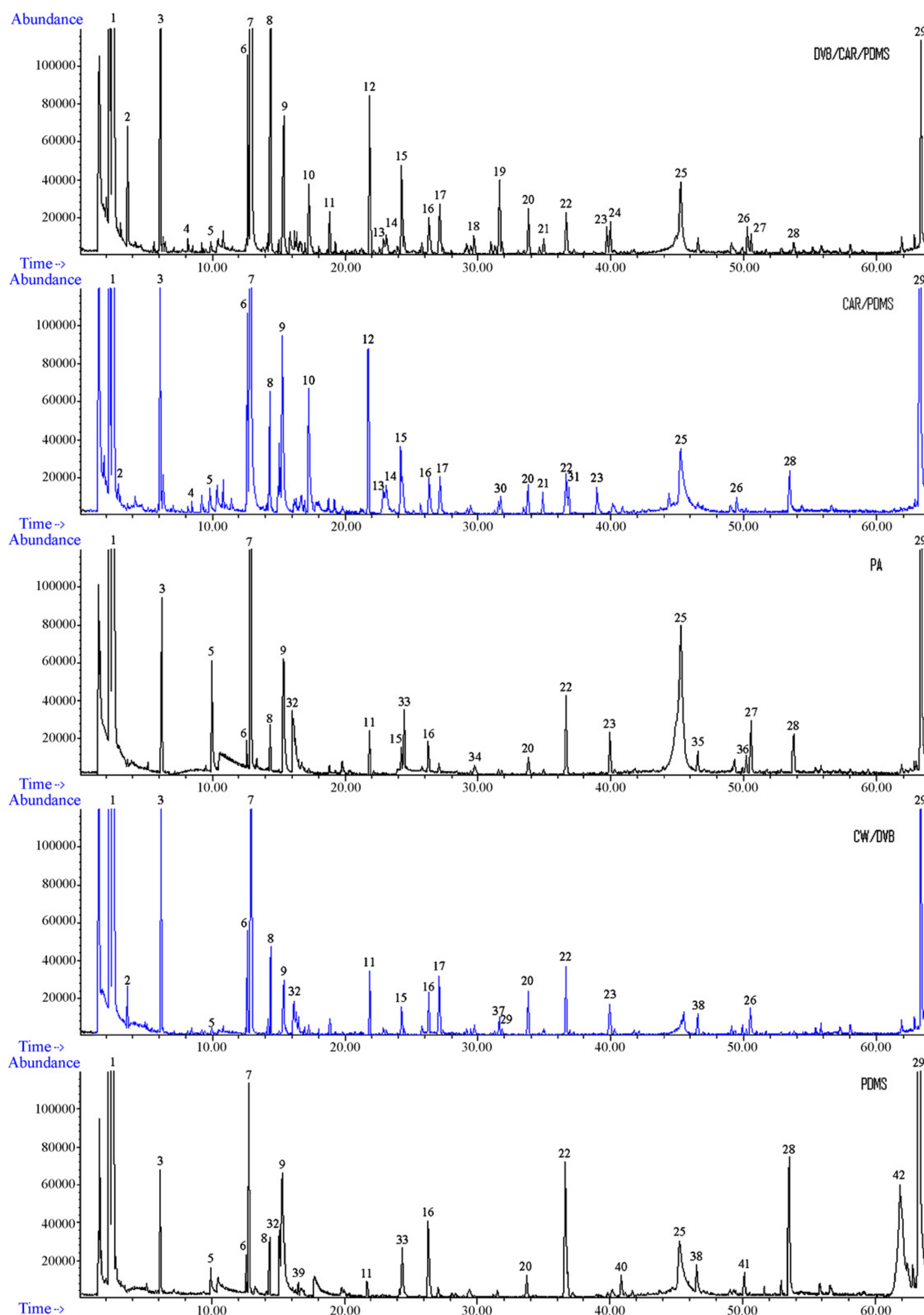


Fig. 1. Chromatograms obtained by SPME analysis of H1 honey with different fibres using in the headspace sampling mode with 30% NaCl and at 60 °C during 40 min. Peak identification: (1) ethanol; (2) toluene; (3) 4-methyl-2-pentanol (IS); (4) 1,3-butanediol; (5) 1-hydroxy-2-propanone; (6) nonanal; (7) 3-octanol (IS); (8) *trans*-linalool oxide; (9) furfural; (10) benzaldehyde; (11) linalool; (12) hotrienol; (13) benzeneacetaldehyde; (14) acetophenone; (15) 1-nonanol; (16) eicosane; (17) naphthalene; (18) 3-ethyl-2-pentene; (19) 2-methyl-2-phenylethyl ester propanoic acid; (20) *E*-6,10-dimethyl-5,9-undecadien-2-one; (21) α-2-propenyl-benzenemethanol; (22) heptadecane; (23) 2-ethyl-hexanoic acid; (24) 1-ethyl-2-hepthyl-cyclopropane; (25) 1,3-dihydroxy-2-propanone; (26) nonanoic acid; (27) *N*-(4-hydroxyphenyl)acetamide; (28) 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4one; (29) 5-hydroxymethylfurfural; (30) α-methyl benzenemethanol; (31) α-ionol; (32) acid acetic; (33) furanmethanol; (34) 2-hydroxy-2-cyclopenten-1-one; (35) tridecane; (36) acetoxymethyl-2-furaldehyde; (37) ethyl ester benzenepropanoic acid; (38) nonadecane; (39) hexadecane; (40) furyl hydroxymethyl ketone; (41) 5-acetoxymethylfurfural; (42) 4-(2-hydroxyethyl)phenol.

Table 1
Volatile compounds identified in H1 honey after dynamic headspace solid-phase microextraction using different coatings (extraction temperature: 60 °C; extraction time: 40 min)

RT (min)	KI[a]	Compound	Identity[b]	SPME coating				
				PA	CW/DVB	CAR/PDMS	PDMS	DVB/CAR/PDMS
2.575	929	Ethanol	A, B	x	x	x	x	x
3.614	1023	Toluene	A, B		x			x
4.186	1064	Hexanal	A, B			x		
6.295	1178	Heptanal	A, B			x		x
8.259	1254	1,3-Butanediol	A					x
8.481	1261	1-Methyl-3-(1-methylethyl)-benzene	A, B	x		x		x
9.233	1285	2,4,6,8-Tetramethyl-1-undecane	B	x		x		
9.942	1307	1-Hydroxy-2-propane	B	x		x	x	x
10.864	1337	Linalool	B	x		x		
12.668	1389	Nonanal	A, B	x	x	x		x
12.796	1392	(*E*)-2-Nonen-1-ol	A, B				x	x
14.130	1432	Heptanoic acid ethyl ester	B	x		x		x
14.274	1436	1,4-Diclorobenzene	A, B	x	x	x		x
14.401	1439	*Trans*-linalool oxide	B	x	x	x	x	x
15.025	1457	Acetic acid	B		x		x	x
15.368	1466	*Cis*-linalool oxide	B		x			
15.368	1466	Furfural	A, B	x		x	x	x
15.767	1477	α-Cubebeno	A, B					x
16.149	1486	Methyl ester nonanoic acid	A, B	x		x		
16.578	1497	Hexadecane	B		x			x
16.764	1502	1-(2-Furanyl)-ethanone	A, B	x		x		x
17.009	1508	TBH[c]	B		x			x
17.009	1508	(*E*,*E*,*E*)-2,4,6-Octatriene	A, B	x		x		
17.300	1515	Benzaldehyde	A, B		x	x		x
18.849	1549	(*S*)-Linalool	B		x			x
18.849	1549	5-Methyl-1,3,6-heptatriene	A, B	x		x		
19.248	1558	3-Methyl-1,5-pentenediol	B					x
19.248	1558	1-Hexene	A, B	x		x		
19.801	1569	5-Methylfurfural	B					x
20.052	1574	β-Elemene	B		x			
21.343	1600	α-4-Dimethyl-3-cyclohexene-1-acetaldehyde	B					x
21.840	1611	Hotrienol	B	x	x	x	x	x
22.144	1617	Dihydro-*cis*-α-copaene-8-ol	B					x
22.589	1626	Pulegone	B					x
22.893	1633	Phenylacetaldehyde	B	x	x	x		x
23.122	1637	Acetophenone	B	x	x	x		x
23.475	1644	3-Carene	A, B					x
23.981	1654	1-Nonanol	A, B	x	x	x		x
24.252	1660	2-Furanmethanol	A, B	x	x	x	x	x
25.472	1683	D-Germacrene	B					x
26.312	1698	Heptadecane	B	x	x	x	x	x
27.090	1714	Naphthalene	A, B	x	x	x		x
28.038	1733	β-Cubebene	A, B					x
29.198	1756	Methyl ester 2-hidroxy-benzoic acid	A,B					x
29.368	1759	*Cis*-α-bisabolene	A, B					x
29.368	1759	α-Ocimene	B		x			
29.734	1766	(*Z*)-Heptene	A, B					x
31.591	1801	DPEPA[d]	B					x
31.591	1801	β-Damascenone	A, B		x			
31.591	1801	TMCHB[e]	B	x		x		
31.819	1805	α-Methyl-benzenemethanol	A, B	x	x	x		x
33.474	1839	Ethyl ester dodecanoic acid	B	x		x		
33.774	1845	(*E*)-6,10-Dimethyl-5,9-undecadien-2-one	B	x	x	x	x	x
35.010	1869	α-2-Propenyl-benzenemethanol	B	x		x		x
36.628	1898	Nonadecane	B	x	x	x	x	x
36.918	1904	α-Ionol	A, B				x	
36.918	1904	DDMMP[f]	A, B					x
40.004	1967	2-Ethyl-hexanoic acid	A, B		x			
42.142	2008	2-Methyl-pentanal	B		x			
45.000	2068	1,3-Dihydroxy-2-propanone	A, B	x		x	x	x
46.548	2098	10-Methyl eicosane	B		x			x

Table 1 (*Continued*)

RT (min)	KI[a]	Compound	Identity[b]	SPME coating				
				PA	CW/DVB	CAR/PDMS	PDMS	DVB/CAR/PDMS
49.500	2162	Nonanoic acid	B	x	x	x		x
53.494	2225	DDMP[g]	A, B	x		x	x	x
55.814	2251	2,3,4-Trimethyl-hexane	A, B				x	x
58.057	2275	2-Acetylbenzoic acid	B		x			
61.825	2345	4-Hydroxy-benzenmethanol	B				x	
63.381	2395	5-Hydroxymethylfurfural	B	x		x	x	x
64.113	2418	2,6-Dimethoxy-4-(2-propenyl)-phenol	B		x			
65.352	2457	Dihydro-4-hydroxy-2(3*H*)-furanone	A, B					x
Total compounds identified by fibre				32	30	35	17	50

[a] Experimentally determined Kovàts indices on the BP-20 column, relative to C_8–C_{20} hydrocarbons.
[b] (A) Components identified on the basis of the retention time and EI mass spectra of pure standard; (B) components identified on the basis of their EI mass spectra only.
[c] 1,7,7-Trimethyl-bicyclo[2.2.1]hepten-2-ene.
[d] 2,2-Dimethyl-2-phenylethyl ester propanoic acid.
[e] 1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one.
[f] 4,6-Di(1,1-dimethylethyl)-2-methylphenol.
[g] 2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4one.

Each type of honey showed a typical composition. H1 honey (Fig. 3) is characterized by a high amount of ethanol, furfural, nonanal, 1,3-dihydroxy-2-propanone, *trans*-linalool oxide, benzaldehyde, hotrienol, and nonanol. Some identified compounds are specific of this sample. Therefore, heptanal, ethyl heptanoate, α- and β-cububene, (*Z*)-heptene, α-propenyl-2-benzenemethanol, pulegone, acetophenone, 3-carene, D-germacrene and 2,3,4-trimethylhexane, were identified only in this honey sample. This sample is also characterized by the lowest level of 5-hydroxymethylfurfural.

H2 (Fig. 3) is characterized by a high amount of hotrienol. This compound was identified in each honey analysed but at lower levels. Also 5-hydroxymethylfurfural, benzeneacetaldehyde, furfural and *trans*-linalool oxide, are present in significantly high amounts, when compared with the H1, H3 and H4 honey samples. 1,3,8-*p*-menthatriene, 1-ethyl-2-methylcyclopropane, cycloheptane, and β-phenylethanol, are present only in H2 honey type.

In H3 honey were identified 60 volatile compounds. As shows Fig. 3, the most intense peaks correspond to *o*-methoxyacetophenone, benzeneacetaldehyde, hotrienol, furfural, ethyl dodecanoate and 2-ethyl hexanoic acid. *o*-methoxyacetophenone and 2-ethyl hexanoic acid can be used to differentiate these honey type from H1, H2 and H4 samples, since were detected, at high levels, only in this sample. 5-Hydroxymethylfurfural, 1,3-dihydroxy-2-propanone, *trans*-linalool oxide, furfural, hotrienol and benzeneacetaldehyde, are the dominating components found in the H4 sample. Forty-eight compounds were positively identified in this honey with the experimental conditions used. 3-Methyl-1-butanol, (*E*)-2-hexen-1-ol, 3-methyl-1-pentanol, 5-acetoxymethylfurfural and

Table 2
Sorption capacity of different fibres for extraction of H1 honey volatile compounds during dynamic HS-SPME extraction, expressed as peak area (40 min at 60 °C with salt saturation)

Class of compounds	SPME fibre				
	PA	CW/DVB	DVB/CAR/PMS	PDMS	CAR/PDMS
Higher alcohols	1.10×10^8	1.12×10^8	8.07×10^7	5.34×10^7	1.00×10^8
Fatty acids	7.78×10^6	3.53×10^6	1.23×10^6	8.04×10^5	5.71×10^5
Ethyl esters	–	–	2.85×10^6	–	6.70×10^5
NT[a]	2.16×10^6	6.08×10^6	8.95×10^6	1.99×10^6	8.34×10^6
Sesquiterpenoids	–	5.21×10^5	1.63×10^6	–	–
Furanic compounds	1.04×10^8	1.43×10^7	7.16×10^6	1.33×10^7	3.60×10^7
Carbonyl compounds	4.71×10^7	3.05×10^6	1.19×10^7	5.75×10^5	1.91×10^7
HSHAr[b]	2.98×10^6	7.57×10^6	1.38×10^7	5.06×10^6	5.67×10^6
Miscellaneous	1.95×10^6	–	5.64×10^5	–	3.10×10^5
Sum	2.04×10^8	1.63×10^8	2.84×10^8	9.09×10^7	1.71×10^8
% R.S.D. ($n = 4$) on sum	4.66	4.89	2.81	1.67	11.63

[a] C_{13}-norisoprenoids and monoterpenes.
[b] Aliphatic and aromatic hydrocarbons.

Table 3
Volatile compounds identified in different types of Portuguese honeys after dynamic headspace solid-phase microextraction using DVB/CAR/PDMS coating (extraction temperature: 60 °C; extraction time: 40 min)

RT (min)	KI[a]	Compound	RPA (%)				RPC (%)			
			H1	H2	H3	H4	H1	H2	H3	H4
2.575	929	Ethanol	56.16	41.86	44.27	29.30	51	69	100	98
3.228	992	Tricloromethane	–	–	1.25	–	–	–	100	–
3.614	1023	Toluene	3.99	2.95	2.32	1.61	48	72	80	100
5.941	1162	2,6-Dimethyl-4-heptanone	–	–	–	0.09	–	–	–	100
6.295	1178	Heptanal	0.60	–	–	–	100	–	–	–
7.105	1212	3-Methyl-1-butanol	–	–	–	0.16	–	–	–	100
8.187	1252	3-Octanone	–	0.35	–	0.10	–	100	–	80
8.252	1254	Styrene	–	–	0.16	–	–	–	100	–
8.259	1254	1,3-Butanediol	0.25	–	–	–	100	–	–	–
8.481	1261	1-Methyl-2-(1-methylethyl)-benzene	0.19	0.14	0.13	0.12	35	41	58	100
9.377	1289	1-Methoxy-2-methyl-propane	–	–	–	0.26	–	–	–	100
9.942	1307	1-Hydroxy-2-propane	0.95	0.54	–	0.22	100	55	–	96
10.387	1322	2-Heptanol	–	–	–	0.14	–	–	–	100
12.524	1385	Methyl ester octanoic acid	–	–	0.49	0.15	–	–	100	57
12.668	1389	Nonanal	6.94	–	–	–	100	–	–	–
12.629	1388	(*E*)-2-Hexen-1-ol	–	–	–	0.36	–	–	–	100
12.796	1392	(*E*)-2-Nonen-1-ol	0.93	1.25	1.55	–	17	53	100	–
14.100	1431	1-Methyl-4-(1-methylethenyl)-benzene	–	0.67	1.05	0.36	–	43	100	64
14.13	1432	Heptanoic acid ethyl ester	0.20	–	–	–	100	–	–	–
14.267	1436	1,4-Dichloro-benzene	1.05	–	–	–	100	–	–	–
14.401	1439	*Trans*-linalool oxide	4.99	7.83	4.92	3.90	30	73	69	100
14.469	1441	1,3,8-*p*-Menthatriene	–	0.39	–	–	–	100	–	–
15.025	1457	Acetic acid	1.12	2.11	0.90	0.78	35	99	66	100
15.368	1466	Furfural	8.09	11.31	6.10	3.62	32	100	82	88
15.767	1477	α-Cubebene	1.67	–	–	–	100	–	–	–
16.13	1486	Methyl ester nonanoic acid	–	–	0.55	0.13	–	–	100	43
16.241	1489	2-Ethyl-1-hexanol	–	–	0.29	–	–	–	100	–
16.358	1492	Decanal	–	0.86	0.31	0.14	–	100	53	45
16.535	1496	Benzofuran	–	–	0.85	–	–	–	100	–
16.578	1497	Hexadecane	0.34	–	–	0.19	29	–	–	100
16.764	1502	1-(2-Furanyl)-ethanone	0.20	0.92	0.60	0.27	14	100	98	80
16.917	1505	1-(1,4-Dimethyl-3-cyclohexen-1-yl)-ethanone	–	–	0.41	0.18	–	–	100	79
17.009	1508	1,7,7-Trimethyl-bicyclo[2.2.1]hepten-2-ene	0.35	–	–	–	100	–	–	–
17.3	1515	Benzaldehyde	4.60	2.80	0.80	–	73	100	44	–
17.47	1518	2-Nonanol	–	–	0.76	–	–	–	100	–
18.009	1531	Ethyl ester octanoic acid	–	–	0.34	–	–	–	100	–
18.849	1549	(*S*)-Linalool	1.46	0.78	1.16	0.56	51	44	100	88
19.189	1557	*Cis*-1-ethyl-2-methyl-cyclopropane	–	0.37	–	–	–	100	–	–
19.192	1557	(*S*)-(+)-3-Methyl-1-pentanol	–	–	–	0.17	–	–	–	100
19.801	1569	5-Methylfurfural	0.32	0.5	0.32	–	28	99	100	–
20.774	1589	2-Methyl benzofuran	–	–	0.86	–	–	–	100	–
21.343	1600	α-4-Dimethyl-3-cyclohexene-1-acetaldehyde	0.10	0.84	–	0.17	21	100	–	55
21.840	1611	Hotrienol	3.79	30.33	7.20	4.98	8	100	36	45
22.144	1617	Dihydro-*cis*-α-copaene-8-ol	0.02	–	–	–	100	–	–	–
22.562	1626	1-Ethenyl-4-methoxybenzene	–	–	0.72	–	–	–	100	–
22.589	1626	Pulegone	0.14	–	–	–	100	–	–	–
22.893	1633	Phenylacetaldehyde	0.50	12.44	7.92	3.69	2	100	89	82
23.122	1637	Acetophenone	1.24	–	–	–	100	–	–	–
23.475	1644	3-Carene	0.29	–	–	–	100	–	–	–
23.661	1648	2-Hydroxy-3,5,5-trimethyl-cyclohex-2-enone	–	0.87	–	0.50	–	68	–	100
24.252	1660	1-Nonanol	4.04	1.18	1.00	0.66	100	60	83	99
24.436	1663	2-Furanmethanol	1.52	1.51	–	1.32	27	36	–	100
25.105	1676	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	–	0.38	0.55	0.27	–	60	100	89
25.472	1683	D-Germacrene	0.60	–	–	–	100	–	–	–
25.681	1687	1-Methyl-4-(1-methylethylidene)-cyclohexene	–	–	0.20	–	–	–	100	–
25.703	1687	2,6-Dimethyl-5,7-octadien-2-ol	–	0.14	–	0.11	–	10	100	16
26.272	1697	Eicosane	–	1.22	1.26	–	–	100	95	–
26.312	1698	Heptadecane	2.40	–	–	0.64	84	–	–	100
27.011	1712	8-Heptadecane	–	–	0.29	–	–	–	100	–
27.090	1714	Naphthalene	4.45	–	0.14	–	100	–	11	–
27.318	1719	2-Heptadecanol	–	–	0.78	–	–	–	100	–

Table 3 (*Continued*)

RT (min)	KI[a]	Compound	RPA (%)				RPC (%)			
			H1	H2	H3	H4	H1	H2	H3	H4
27.328	1719	2-Tetradecanol	–	0.26	–	–	–	100	–	–
27.357	1720	1,2-Dihydro-3,6,8-trimethyl-naphthalene	–	0.46	–	–	–	100	–	–
28.038	1733	β-Cubebene	0.35	–	–	–	100	–	–	–
29.172	1756	2-Hydroxy methyl ester benzoic acid	–	2.02	–	0.36	–	100	–	48
29.198	1756	Methyl ester 2-hidroxy-benzoic acid	0.52	–	–	–	100	–	–	–
29.368	1759	*Cis*-α-bisabolene	0.25	–	–	–	100	–	–	–
29.446	1761	Cycloheptane	–	0.56	–	–	–	100	–	–
29.453	1761	2-Methyl-cyclopentanone	–	–	–	0.33	–	–	–	100
29.734	1766	(*Z*)-Heptene	0.96	–	–	–	100	–	–	–
30.400	1779	4-Hydroxy-acetophenone	–	–	0.25	–	–	–	100	–
31.182	1793	Methyl ester dodecanoic acid	–	–	0.81	–	–	–	100	–
31.587	1800	2-Phenylethyl ester propanoic acid	–	–	0.33	0.30	–	–	61	100
31.591	1801	2,2-Dimethyl-2-phenylethyl ester propanoic acid	4.99	–	–	–	100	–	–	–
31.819	1805	(*S*)-α-Methyl-benzenemethanol	0.57	–	0.42	–	55	–	100	–
33.395	1837	Ethyl ester dodecanoic acid	–	1.38	6.80	–	–	15	100	–
33.424	1838	Ethyl ester decanoic acid	–	–	–	0.22	–	–	–	100
33.594	1841	α-4-Trimethyl-benzenemethanol	–	–	–	0.11	–	–	–	100
33.774	1845	(*E*)-6,10-Dimethyl-5,9-undecadien-2-one	2.15	–	0.96	–	91	–	100	–
34.336	1856	3-Methylbuthyl ester pentadecanoic acid	–	–	0.24	–	–	–	100	–
34.901	1866	Phenol	–	–	0.64	–	–	–	100	–
35.010	1869	α-2-Propenyl-benzenmethanol	0.78	–	–	–	100	–	–	–
36.565	1897	β-Phenylethanol	–	1.84	–	–	–	100	–	–
36.628	1898	Nonadecane	2.53	–	1.90	0.89	54	–	100	85
36.830	1902	α-Ionol	–	–	0.56	–	–	–	100	–
36.918	1904	4,6-Di-(1,1-dimethylethyl)-2-methyl phenol	1.80	–	–	–	100	–	–	–
37.052	1907	Benzyl nitrile	–	–	0.26	0.16	–	–	91	100
38.602	1939	2,3,4,5-Tetramethyl-1,4-hexadiene	–	–	–	0.97	–	–	–	100
38.951	1946	2-Ethyl hexanoic acid	–	–	6.08	–	–	–	100	–
40.203	1971	*o*-Methoxyacetophenone	–	–	19.25	–	–	–	100	–
40.664	1979	MFPOE[b]	–	–	–	0.29	–	–	–	100
40.906	1984	Furyl hydroxymethyl ketone	–	–	–	0.41	–	–	–	100
41.71	1999	Methyl tetradecanoate	–	–	0.48	–	–	–	100	–
43.727	2042	Ethyl ester tetradecanoic acid	–	–	0.55	–	–	–	100	–
44.358	2055	Octanoic acid	–	0.81	1.61	0.68	–	46	100	78
45.355	2075	1,3-Dihydroxy-2-propanone	6.20	6.97	5.61	6.90	18	53	45	100
46.548	2098	10-Methyleicosane	0.75	–	–	–	100	–	–	–
49.862	2170	Cyclododecane	–	–	0.42	–	–	–	100	–
50.189	2176	5-Acetoxymethylfurfural	–	–	–	0.29	–	–	–	100
50.679	2186	Nonanoic acid	0.70	0.75	1.01	0.34	26	57	100	63
51.673	2204	15-Methyl ester hexadecanoic acid	–	–	0.40	–	–	–	100	–
53.461	2225	Ethyl ester hexadecanoic acid	–	–	0.72	–	–	–	100	–
53.824	2229	DDMP[c]	1.61	2.23	0.70	1.61	24	57	25	100
54.464	2236	Ethyl ester *E*-11 hexadecanoic acid	–	–	1.02	–	–	–	100	–
54.732	2239	2,3-Dimethoxy-naphthalene	–	–	1.67	–	–	–	100	–
55.814	2251	2,3,4-Trimethyl-hexane	6.06	–	–	–	100	–	–	–
59.217	2287	3,5-Dimethoxy methyl ester benzoic acid	–	–	0.82	–	–	–	100	–
63.074	2345	Dodecanoic acid	–	–	1.12	–	–	–	100	–
63.381	2395	5-Hydroxymethylfurfural	0.31	14.71	4.86	10.93	1	59	29	100
65.352	2457	Dihydro-4-hydroxy-2-(3*H*)-furanone	0.33	–	–	0.37	21	–	–	100
Total compounds identified by honey							50	37	60	48
Sum of total area	2.8×10^8	4.3×10^8	5.2×10^8	5.12×10^8						
% R.S.D. ($n = 4$)	2.81	1.95	7.35	7.30						

[a] Experimentally determined Kovàts indices on the BP-20 column, relative to C_8–C_{20} hydrocarbons.

[b] 1-[3-[2-Methyl-2-[5-methyl-2-furanyl]propyl]oxiranyl]-ethanone.

[c] 2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one.

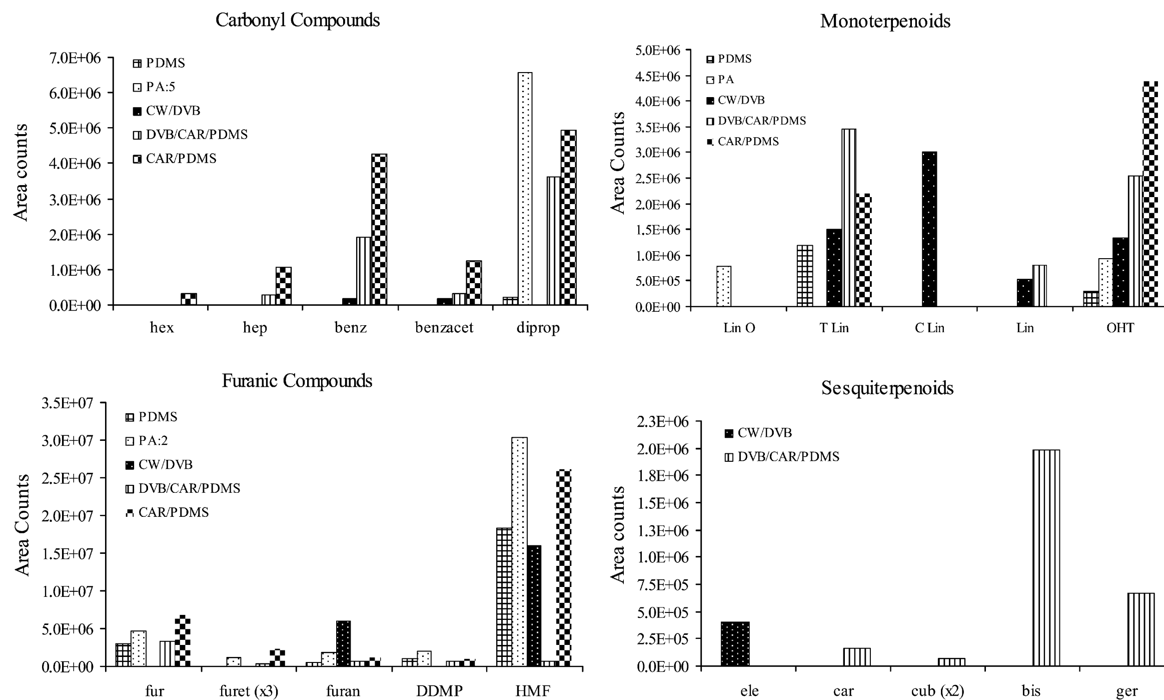


Fig. 2. Comparison of the performance of different SPME coatings on the carbonyl compounds, monoterpenoids, furanic compounds and sesquiterpenoids extraction (hex: hexane; hep: heptane; benz: benzaldehyde; benzacet: benzeneacetaldehyde; diprop: 1,3-hydroxy-2-propanone; Lin O: linalool oxide; T Lin: *trans*-linalool oxide; C Lin: *cis*-linalool oxide; Lin: linalool; OHT: hotrienol; fur: furfural; furet: 1-(2-furanyl)-ethanone; furan: furanmethanol; DDMP: 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one; HMF: 5-hydroxymethylfurfural; ele: β-elemene; car: 3-carene; cub: β-cubebene; bis: α-bisabolene; ger: D-germacrene).

2-methylcyclopentanone, has been detected only in this honey sample. Fig. 4 presents the distribution of compounds classes by honey sample.

The presence of several honey constituents found in this study have been previously reported. The presence of hotrienol in the *Eucryphia lucida* honey [38]. We have also found, for instance aromatic aldehydes such as benzaldehyde and benzeneacetaldehyde, which have been reported as common components of various unifloral honey [39].

3.3. Multivariate analysis

The proposed headspace $SPME_{DVB/CAR/PDMS}$ method was applied to four different commercial honeys. Evidently, the different concentrations of the volatile constituents determined in these samples allow their differentiation. Although the volatile compounds studied showed important data for the characterization of honey samples, the differentiation is quite difficult to establish from the direct observation of Table 3. Multivariate techniques of data analysis represent a powerful statistical tool to explain this differentiation. The total peak area of each chemical group, higher alcohols (HA), fatty acids (FAc), ethyl esters (EEs), C_{13}-norisoprenoids/monoterpenols/sesquiterpenoids (NTS1), furanic compounds (FC), carbonyl compounds (CaC) and aliphatic/aromatic hydrocarbons (HSHAr), were used as variable vectors for multivariate analysis in order to obtain more detailed information. When PCA was applied to the total peak area different chemical classes, three factors were extracted and 98.3% of the total variance was explained (Table 4). As can be seen in Fig. 5a, a clear separation can be observed. Considering the factor loadings of the variables (Table 5), the most influential variables (chemical groups) to the first component (PC1, 50.1%), are carbonyl compounds, ethyl esters and fatty acids, while NTS1, HSHAr (25.9%) and furan derivatives (22.3%), are the variables that most contribute to the PC2 and PC3, respectively. Fig. 5a shows the scores scatter plot of the first two principal components (50.1% of the total variability) that represents the

Table 4
Percentage of variance and percentage of cumulative variance explained by the three first principal components

Component	Total variance explained					
	Extraction sums of squared loadings			Rotation sums of squared loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
1	3.771	53.877	53.877	3.509	50.124	50.124
2	2.449	34.988	88.864	1.814	25.917	76.041
3	0.664	9.491	98.355	1.562	22.314	98.355

Extraction method: principal component analysis.

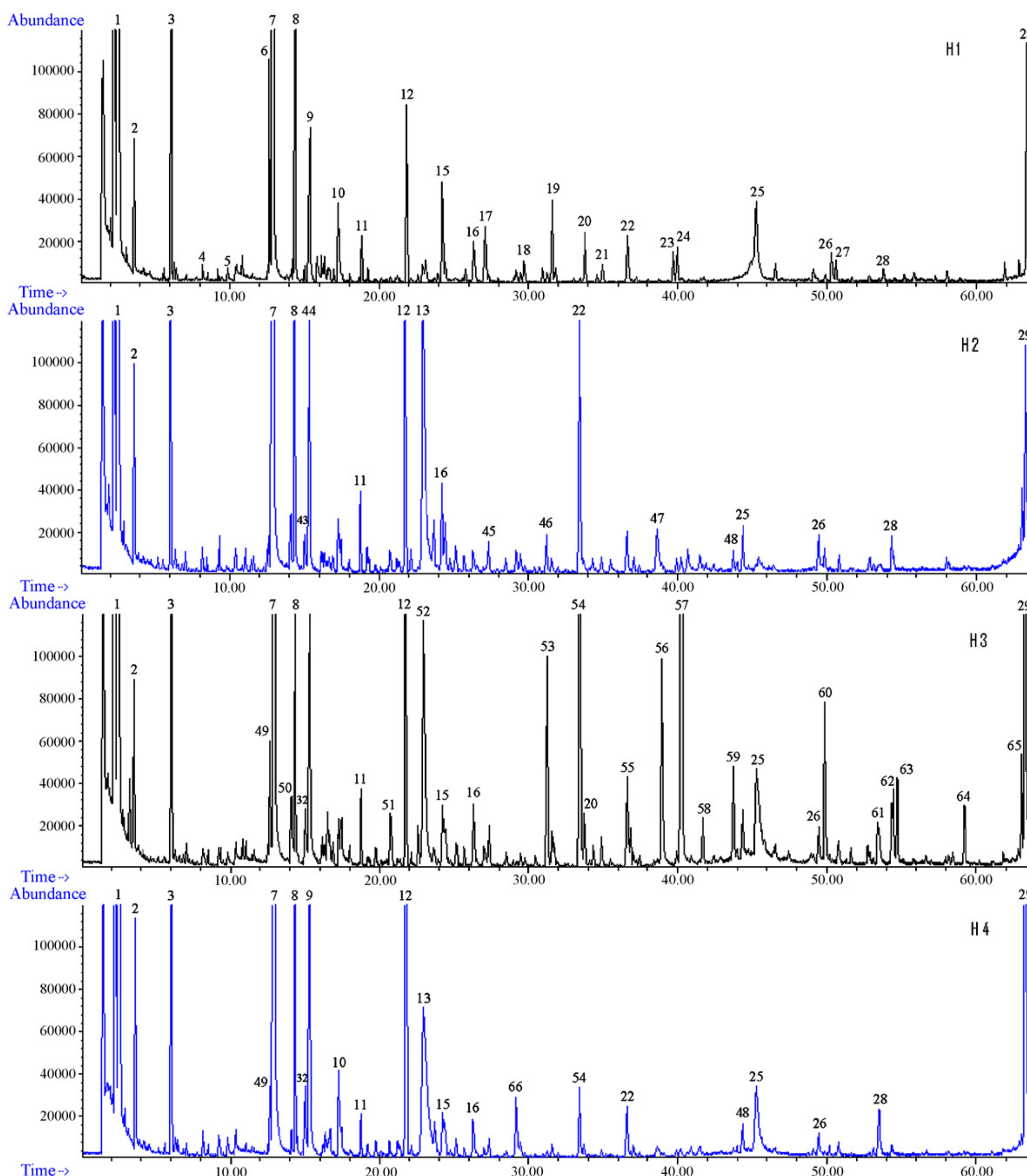


Fig. 3. Comparison of GC–qMS profiles of HS-SPME$_{DVB/CAR/PDMS}$ H1–H4 honey extracts obtained at 60 °C during 40 min. Peak identification: (1) ethanol; (2) toluene; (3) 4-methyl-2-pentanol (IS); (4) 1,3-butanediol; (5) 1-hydroxy-2-propanone; (6) nonanal; (7) 3-octanol (IS); (8) *trans*-linalool oxide; (9) furfural; (10) benzaldehyde; (11) linalool; (12) hotrienol; (13) benzeneacetaldehyde; (14) acetophenone; (15) 1-nonanol; (16) eicosane; (17) naphthalene; (18) 3-ethyl-2-pentene; (19) 2-methyl-2-phenylethyl ester propanoic acid; (20) *E*-6,10-dimethyl-5,9-undecadien-2-one; (21) α-2-propenyl-benzenemethanol; (22) heptadecane; (23) 2-ethyl-hexanoic acid; (24) 1-ethyl-2-hepthyl-cyclopropane; (25) 1,3-dihydroxy-2-propanone; (26) nonanoic acid; (27) *N*-(4-hydroxyphenyl)acetamide; (28) 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one; (29) 5-hydroxymethylfurfural; (31) α-ionol (32) acid acetic; (33)–(42) (compounds from 33 to 42 were not detected with this fibre); (43) 1-methyl-4-(1-methylethyl)-benzene; (44) *cis* linalool oxide; (45) 2-undecanol; (46) 2-methyl-cyclopentanone; (47) 2,3,4,5-tetramethyl-1,4-hexadiene; (48) octanoic acid; (49) 2-nonen-1-ol; (50) ethyl ester octanoic acid; (51) methyl ester decanoic acid; (52) ethyl ester decanoic acid; (53) methyl ester dodecanoic acid; (54) ethyl ester dodecanoic acid; (55) phenol; (56) 2-ethyl hexanoic acid; (57) *o*-methoxyacetophenone; (58) methyl tetradecanoate; (59) ethyl ester tetradecanoic acid; (60) cyclododecane; (61) ethyl ester hexadecanoic acid; (62) ethyl ester hexadecanoic acid E11; (63) 5,7,8-trimethyl coumarin; (64) 3,5-dimethoxy-methyl ester benzoic acid; (65) dodecanoic acid; (66) 2-hydroxy methyl ester benzoic acid.

distinction among the honey samples. Fig. 5b represents the corresponding loadings plot that established the relative importance of each chemical group. H1 and H2 honeys (third quadrant) are characterized by the carbonyl compounds (CaC), ethyl esters (EEs) and in lower extent, by fatty acids (FAc). The H3 honey is related to the positive PC1 side. Higher alcohols, is the variable which characterize them. H4 honey represented in the second quadrant being characterized by carbonyl compounds (CaC) and fatty acids (FAc) (Fig. 5b).

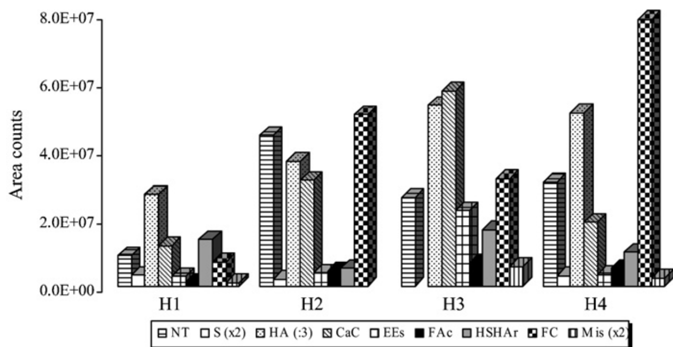


Fig. 4. Distribution of compounds classes by honey samples (NT: C_{13}-norisoprenoids and monoterpenoids; S: sesquiterpenoids; HA: higher alcohols; CaC: carbonyl compounds; EEs: ethyl esters; FAc: fatty acids; HSHAr: aliphatic and aromatic hydrocarbons; FC: furanic compounds; Mis: miscellaneous).

Table 5
Loadings of volatiles in the first three principal components

	Rotated component matrix[a]		
	Component 1	Component 2	Component 3
CC	0.994	0.081	−0.019
EE	0.960	−0.220	−0.060
FA	0.783	0.208	0.579
Alc	0.781	−0.169	0.556
NTS	0.186	0.938	0.284
HSHA	0.579	−0.802	−0.124
F	−0.057	0.403	0.904

(1) PC1; (2) PC2 and (3) PC3; rotation method: Varimax with Kaiser normalization; CC: carbonyl compounds; EE: ethyl esters; FA: fatty acids; Alc: higher alcohols; NTS: C_{13}-norisoprenoids, monoterpenoids and sesquiterpenoids; HSHA: aliphatic and aromatic hydrocarbons; F: furanic compounds. Extraction method: principal component analysis. Rotation method: Varimax with Kaiser normalization.

[a] Rotation converged in five iterations.

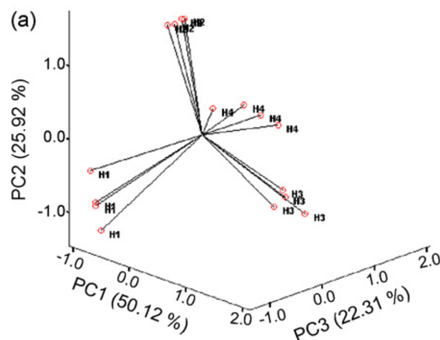
After PCA, a linear discriminant analysis (LDA) was run, using the above-mentioned variables, in order to obtain suitable classification rules. Fig. 6 shows a projection of the honeys in two-dimensional space, generated by the two first discriminate functions that explain 97.9% of the total variance. Four groups representing each honey, H1–H4, were clearly observed. The good agreement achieved indicates that very acceptable classification functions can be deduced. The leave one out method was used as cross-validation procedure to evaluate the classification performance (Table 6).

Table 6
Prediction abilities for the different honeys, using stepwise discriminant analysis

	HM	Classification results[a,b]				
		Predicted group membership				Total
		H1	H2	H3	H4	
Original						
Count	H1	4	0	0	0	4
	H2	0	4	0	0	4
	H3	0	0	4	0	4
	H4	0	0	0	4	4
%	H1	100.0	0	0	0	100.0
	H2	0	100.0	0	0	100.0
	H3	0	0	100.0	0	100.0
	H4	0	0	0	100.0	100.0
Cross-validated[c]						
Count	H1	4	0	0	0	4
	H2	0	4	0	0	4
	H3	0	0	4	0	4
	H4	0	0	0	4	4
%	H1	100.0	0	0	0	100.0
	H2	0	100.0	0	0	100.0
	H3	0	0	100.0	0	100.0
	H4	0	0	0	100.0	100.0

[a] 1000% of original grouped cases correctly classified.
[b] 1000% of cross-validated grouped cases correctly classified.
[c] Cross-validation is done only for those cases in the analysis. In cross-validation, each case is classified by the functions derived from all cases other than that case.

From the results it can be concluded that headspace SPME coupled to GC–qMS and chemometrics is a very appropriate sampling technique to distinguish the different Portuguese honeys studied based on their volatile profile.



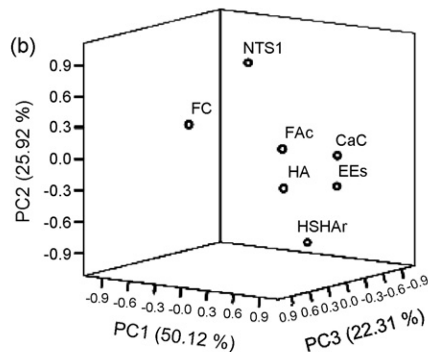


Fig. 5. PC1 and PC2 scatter plot of the main sources of variability between honey samples (H1–H4). (a) Distinction between the samples (scores); (b) relation between the chemical classes (loadings); Variables identification: CaC: carbonyl compounds; EEs: ethyl esters; FAc: fatty acids; HA: higher alcohols; NTS1: C_{13}-norisoprenoids/monoterpenes/sesquiterpenoids; HSHAr: aliphatic and aromatic hydrocarbons; FC: furanic compounds.

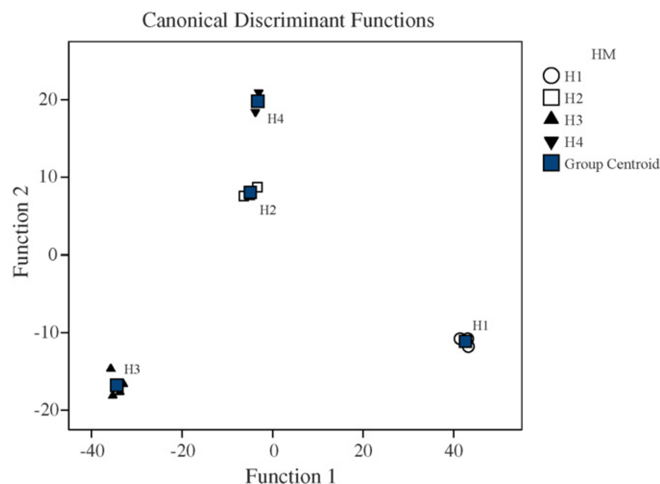


Fig. 6. Differentiation between H1–H4 honeys by applying LDA.

4. Conclusions

Headspace solid-phase microextraction sampling followed by GC–qMS analysis provides an appropriate and selective way to characterize the volatile compounds in honey. Is a simple procedure of extraction with a great capacity of concentration and combines extraction to a rapid, sensitive and solvent-free method suitable for determination of volatile and semivolatile compounds. The chromatographic profiles obtained after extraction with PDMS, PA, CAR/PDMS, CW/DVB and DVB/CAR/PDMS coatings suggest that the later was the most suitable for the SPME analysis of honey volatiles. The more polar fibre, PA, shows no more effective extraction for polar such as higher alcohols, fatty acids, furan derivatives and carbonyl compounds, while DVB/CAR/PDMS favours the extraction of less polar compounds like ethyl esters, C_{13}-norisoprenoids/monoterpenes, sesquiterpenoids and aliphatic and aromatic hydrocarbons. More than 100 volatile compounds, including higher alcohols, ethyl esters and fatty acids, were identified. The higher alcohols, fatty acids and C_{13}-norisoprenoids/monoterpenes constitute important aroma groups compounds which contributes with “fruity”, “cheese/fatty” and “flowery” notes to honey sensory properties. Using a chemometric approach (PCA and LDA), the volatile composition in terms of average peak areas, provides a suitable tool to differentiate between the analysed honeys.

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