Food Chemistry 124 (2011) 401–410

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Contribution to the characterisation of honey-based Sardinian product *abbamele*: Volatile aroma composition, honey marker compounds and antioxidant activity

I. Jerković [a,*], A. Kasum [a], Z. Marijanović [b], C.I.G. Tuberoso [c]

[a] Department of Organic Chemistry, Faculty of Chemistry and Technology, University of Split, N. Tesle 10/V, 21000 Split, Croatia
[b] Department of Food Technology, Marko Marulić Polytechnic in Knin, Petra Krešimira IV 30, 22300 Knin, Croatia
[c] Department of Toxicology, University of Cagliari, via Ospedale 72, 09124 Cagliari, Italy

ARTICLE INFO

Article history:
Received 17 December 2009
Received in revised form 11 May 2010
Accepted 12 June 2010

Keywords:
Abbamele
Headspace solid-phase microextraction (HS-SPME)
Ultrasonic solvent extraction (USE)
GC and GC–MS
DPPH and FRAP assay
HPLC-DAD

ABSTRACT

Sardinian *abbamele* is a typical product obtained from the honey recuperation from combs (traditional procedure) or by concentration of the honey diluted in water (industrial procedure). Seven *abbamele* samples were obtained to study the volatiles' composition, the presence of honey marker compounds and their relationship with the production procedures. The long thermal treatment applied in *abbamele* production caused very high (1007.0–4405.8 mg/kg) HMF content (HPLC-DAD), while glucose and fructose amounts were quite similar to the honey ones (HPLC-RI). Total antioxidant activity (FRAP assay) of the samples ranged between 13.3 and 71.2 mmol Fe^{2+}/kg, while antiradical activity (DPPH assay) ranged between 3.8 and 23.3 mmol TEAC/kg. Such high antioxidant values were linearly correlated with total phenol amount (1297.8–4469.5 mg GAE/kg) determined by Folin–Ciocalteau method. Thermally derived furan derivatives and terpenes were abundant among the headspace volatiles (HS-SPME), particularly limonene (0.5–76.0%) that probably originated from citrus rinds' addition during *abbamele* production. GC and GC–MS analyses of USE isolates revealed HMF predominance as well as the honey marker compounds (if/when existing) such as methyl syringate (up to 49.2%), marker of *Asphodelus microcarpus* honey. High isophorone percentage (up to 30.9%) determined by HS-SPME followed by minor percentage of 4-ketoisophorone and norisoprenoids in one sample indicated *Arbutus unedo* L. honey use in the production. HPLC-DAD analysis confirmed the presence of specific honey markers: two samples showed high methyl syringate concentrations (150.4–120.1 mg/kg) while homogentisic acid and other specific markers of *A. unedo* honey were found in one sample. The compared GC–MS and HPLC-DAD data proved to be useful to obtain information about the use of specific honey in the production and to verify citrus addition.

1. Introduction

Globalisation of food-stuff market led to a parallel development of re-discovery and protection of traditional foods. Sardinian *abbamele* is a typical product originally obtained from the recuperation of honey from the combs. In ancient times and poor economies, no food was to be wasted and *abbamele* was perfect filler for sweets (Spiggia, 1997). According to *abbamele* traditional production, honey is extracted from combs and the latter are crumbled and dipped into warm water (40 °C). Then, the emerging wax separates and the remaining liquid (water, some honey and pollen) is heated (up to 100 °C) until a brown, honey-like product is obtained (Spano et al., 2008). Today, *abbamele* is sometimes still prepared in this traditional way, but more and more often it is prepared in industrial way by concentration of honey diluted in water. In both ways of preparation it is common to add peels or pieces of citrus fruits. A previous paper (Spano et al., 2008) reported a first chemical investigation of *abbamele* on typical parameters studied for honey (water content, electrical conductivity, pH, free acidity, invertase activity, 5-hydroxymethyl-2-furaldehyde (HMF), total polyphenols and free amino acids). As expected for a product submitted to heat treatment, invertase activity was very low (less than 1.02 U/kg) while HMF values ranged between 881 and 4776 mg/kg. The studied parameters, although interesting for a preliminary characterisation of this product, were not useful to investigate the production cycle of *abbamele*. In addition, it is stated (Spano et al., 2008) that the value of *abbamele* is usually much higher (up to 10 times) than that of honey, but no information on its useful properties were reported. For instance, honey-based *abbamele* could exhibit antioxidant properties contributing to its high value. It is well known that antioxidant activity is one of the beneficial effects of honey that is greatly influenced by its botanical

* Corresponding author. Tel.: +385 21 329434; fax: +385 21 329461.
E-mail address: igor@ktf-split.hr (I. Jerković).

0308-8146/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2010.06.047

origin (Frankel, Robinson, & Berenbaum, 1998; Schramm et al., 2003) as well as heat treatment (Antony, Han, Rieck, & Dawson, 2000).

The first goal of this work was to isolate volatiles from *abbamele* and to obtain very representative chemical composition of more and less volatile compounds applying solid-phase microextraction (HS-SPME) and ultrasonic solvent extraction (USE). Isolated volatiles were analysed by gas chromatography and mass spectrometry (GC, GC–MS), in order to (i) investigate the volatile aroma compounds of *abbamele* and (ii) find potential compounds useful to obtain information on used honey type. Finding a marker compounds in honey-based product is a powerful tool in the determination of the honey used in the production so that this product can be marked according to the honey origin. In this regard, HPLC-DAD was also applied to analyse the presence of nonvolatile honey marker compounds. The last aim of this research is determination of *abbamele* total phenols' amount (Folin–Ciocalteau assay), antiradical (DPPH assay) and total antioxidant activities (FRAP assay).

2. Materials and methods

2.1. Reagents and honey samples

Diethyl ether, pentane and dichloromethane were purchased from Kemika (HR-Zagreb) and were distilled before usage. Anhydrous $MgSO_4$ and NaCl were obtained from Fluka Chemie (CH-Buchs). Methanol, acetonitrile, 5-hydrohymethylfurfural, methyl syringate, homogentisic acid, gallic acid, ferrous sulphate, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tris-(2-pyridyl)-1,3,5-triazine (TPTZ), Folin–Ciocalteau reagens were obtained from Sigma–Aldrich, Fluka (Milan, Italy). Standard of (±)-2-*trans*, 4-*trans* abscisic acid was purchased from A.G. Scientific, Inc. (San Diego, CA). Fructose, glucose, sucrose, sodium carbonate, ferric chloride and $CuSO_4{\cdot}5H_2O$ were supplied by Carlo Erba (Milan, Italy). All other standards were purchased from Sigma–Aldrich (Biovit d.o.o., Varaždin, Croatia). Ultrapure water (18 mΩ) was distilled and then purified with a Milli-Q Advantage A10 System apparatus (Millipore, Milan, Italy).

This study was carried out on seven *abbamele* samples collected from professional producers in different areas of Sardinia (Italy) during the years 2008–2009. Three samples (1–3) were produced in the traditional way, while other four (4–7) were produced in industrial way (Table 1). All the samples were stored in hermetically closed glass bottles at 4 °C until the analysis.

2.2. Headspace solid-phase microextraction (HS-SPME)

The isolation of headspace volatiles was performed using manual SPME fibre with the layer of polydimethylsiloxane/divinylbenzene (PDMS/DVB) obtained from Supelco Co. (Bellefonte, PA, USA). The coating was 1 cm long. The fibre was conditioned prior to use according to the manufacturer's instructions by inserting into the GC injector port.

For HS-SPME extraction 5 mL of *abbamele*/water solution (1:1 v/v; the ionic strength was increased using saturated NaCl water solution) was placed in 15 mL amber glass vial (volume ratio headspace:solution was 1:1 v/v) and hermetically sealed with PTFE/silicone septa. The vial was maintained in a water bath at 60 °C during equilibration (15 min) and extraction (40 min) and was partially submerged so that the liquid phase of the sample was in the water. All the experiments were performed under constant stirring velocity (1000 rpm) by magnetic stirrer. After sampling, the SPME fibre was withdrawn into the needle, removed from the vial and inserted into the injector (250 °C) of the GC and GC–MS for 6 min where the extracted volatiles were thermally desorbed directly to the GC column.

2.3. Ultrasonic solvent extraction (USE)

Ultrasound-assisted extraction (USE) was performed in an ultrasound cleaning bath (Transsonic Typ 310/H, Germany) by the mode of indirect sonication, at the frequency of 35 kHz at 25 ± 3 °C. Forty grams of *abbamele* were dissolved with 22 mL of distiled water in a 100 mL flask. Magnesium sulphate (1.5 g) was added and each sample was extensively vortexed. Different extraction solvents for USE were separately used for the representative sample: (1) pentane, (2) diethyl ether (on the same batch of *abbamele* after sonication with pentane and removing the pentane extract), (3) a mixture pentane: diethyl ether 1:2 (v/v) and (4) dichloromethane. Sonication was held for 30 min. After sonication, the organic layer was separated by centrifugation and filtered over anhydrous $MgSO_4$. Aqueous layer was returned to the flask and another batch of the same extraction solvent (20 mL) was added and extracted by ultrasound for 30 min. Organic layer was separated as the previous one, filtered over anhydrous $MgSO_4$ and aqueous layer was sonicated third time for 30 min with another batch (20 mL) of the extraction solvent. Joined organic extracts were concentrated up to 0.2 mL by fractional distillation and 1 μL was used for GC and GC–MS analyses. After determination of the most suitable extraction solvents for the representative sample, all the samples of *abbamele* were extracted using selected solvents as previously described.

2.4. Gas Chromatography (GC) and gas chromatography–mass spectrometry (GC–MS)

Gas chromatography analyses were carried out on an Agilent Technologies (Palo Alto, CA, USA) gas chromatograph model 7890A equipped with flame ionisation detector. Chromatographic separations were performed on 30 m × 0.25 mm i.d capillary column HP-5MS (5%-phenyl)-methylpolysiloxane, Agilent J & W GC column) with coating thickness 0.25 μm. The oven was temperature-programmed isothermal from 70 °C for 2 min, then increased to 200 °C, at a rate of 3 °C/min and held isothermal for 15 min. Helium at 1 mL/min was used as the carrier gas. Injector temperature was 250 °C and detector temperature was 300 °C. The injected volume was 1 μL and split ratio was 1:50.

Analyses of volatile compounds by gas chromatography–mass spectrometry were carried out with the Agilent gas chromatograph model 7890A fitted with a mass selective detector model 5975C (Agilent Technologies, Palo Alto, CA, USA). Mass detector worked in the electron impact ionisation mode at 70 eV, the mass range was *m/z* 30–300 and ion source temperature was 280 °C. Volatile compound separation was obtained using the same column and oven temperature programme as previously described for GC.

The individual peaks were identified by comparison of their retention indices (relative to C_9–C_{25} *n*-alkanes for HP-5MS column) to those of authentic samples and literature (El-Sayed, 2007 and references therein), as well as by comparing their mass spectra with the Wiley 275 MS library (Wiley, New York, USA) and NIST02 (Gaithersburg, Germany) mass spectral database. The percentage composition of the samples was computed from the GC peak areas using the normalisation method (without correction factors). The component percentages (Table 2) were calculated as mean values from duplicate GC and GC–MS analyses.

2.5. HPLC-RI analysis

Fructose, glucose and sucrose were detected using a Waters LC (Waters S.p.A., Vimodrone, Milan, Italy) fitted with a multisolvent

Table 1
Characteristics of the *abbamele* samples.

	Sample*						
	1	2	3	4	5	6	7
Type of production	*traditional*	*traditional*	*traditional*	*industrial*	*industrial*	*industrial*	*industrial*
Year of production	2007	2007	2007	2007	2006	2008	2008
Moisture (g/100 g)	19 ± 1.2	26.8 ± 0.1	23.8 ± 0.1	27.7 ± 0.2	16.2 ± 0.3	15.3 ± 0.6	18.3 ± 1.4
Fructose (g/100 g)	38.87 ± 0.79	33.26 ± 0.83	27.89 ± 0.05	31.85 ± 0.98	37.65 ± 0.25	36.38 ± 1.53	30.36 ± 0.15
Glucose (g/100 g)	35.21 ± 0.78	27.17 ± 1.16	27.32 ± 1.99	27.11 ± 0.37	35.44 ± 0.26	35.17 ± 3.19	30.35 ± 2.28
Sucrose (g/100 g)	1.26 ± 0.08	n.d.	n.d.	n.d.	1.11 ± 0.09	n.d.	n.d.
Fructose/ Glucose	1.10	1.22	1.02	1.17	1.06	1.03	1.00
HMF (mg/kg)	1237.2 ± 87.5	1115.2 ± 76.6	1823.6 ± 54.6	1892.9 ± 123	2971.5 ± 13	1007 ± 56.3	4405.8 ± 133.4
Total polyphenols (mg GAE[a] /kg)	1297.8 ± 56.5	1959.8 ± 5.5	1377.6 ± 54.1	2039.0 ± 86.9	4469.5 ± 143.6	1491.2 ± 148.9	3468.2 ± 172.7
Methyl syringate (mg/kg)	150.4 ± 3.6	n.d	120.1 ± 4.1	n.d	n.d	n.d	n.d
Homogentisic acid (mg/kg)	n.d	n.d	n.d	n.d	85.2 ± 2.9	n.d	n.d
Unedone [b](mg/kg)	n.d	n.d	n.d	n.d	112.9 ± 3.5	n.d	n.d
trans,*trans*-abscisic acid (mg/kg)	n.d	n.d	n.d	n.d	125.0 ± 6.2	n.d	n.d
cis,*trans*-abscisic acid (mg/kg)	n.d	n.d	n.d	n.d	127.1 ± 4.3	n.d	n.d
FRAP [c](mmol Fe^{2+}/kg)	16.2 ± 0.1	19.4 ± 0.2	13.3 ± 0.1	24.6 ± 0.5	71.2 ± 0.6	19.9 ± 0.9	36.8 ± 0.7
DPPH [d](mmol TEAC/kg)	3.8 ± 0.3	5.7 ± 0.1	3.8 ± 0.2	7.6 ± 0.2	23.3 ± 0.1	5.1 ± 0.3	12.2 ± 1.2

* Values are means ± SD of triplicate determinations.
[a] GAE: gallic acid equivalent.
[b] dosed using *c*,*t*-ABA calibration curve.
[c] FRAP value is expressed as Fe^{2+} millimolar concentration, obtained from a $FeSO_4$ solution having an antioxidant capacity equivalent to that of the dilution of the *abbamele*.
[d] DPPH value is expressed as TEAC millimolar concentration, obtained from a Trolox solution having an antiradical capacity equivalent to that of the dilution of the *abbamele*.

delivery system 600, a column heater set at 35 °C, an autosampler 717 plus with a 50-μl loop and a refractive index detector Varian 356-LC (Varian, Leinì, TO, Italy). Separation was obtained with a Spherisorb NH_2 column (250 × 4.6 mm, 5 μm, Waters) using water and acetonitrile 20:80 (v/v) as mobile phase at a constant flow rate of 0.8 mL/min. Standard and working solutions were prepared in ultrapure water. Calibration curves were built with the method of external standard, correlating the area of the peaks with the concentration with correlation values ranging from 0.9991 to 0.9998. *Abbamele* samples were homogenised, diluted with ultrapure water, filtered through cellulose acetate GD/X septa (0.45 μm, 25 mm Ø, Whatman, Milan, Italy) and injected in HPLC without any further purification.

2.6. HPLC-DAD analysis

5-Hydrohymethyl-furfural (HMF) and specific markers of typical Sardinian honeys were detected and quantified using an HPLC-DAD method as described in Tuberoso et al. (2010). Briefly, a Varian system ProStar HPLC fitted with a ThermoSeparation diode array detector SpectroSystem UV 6000lp (ThermoSeparation, San Jose, CA, USA) set at 280 nm was employed. Separation was obtained with a Gemini C18 column (150 × 4.60 mm, 3 μm, Phenomenex, Casalecchio di Reno, BO, Italy) using 0.2 M phosphoric acid (solvent A) and acetonitrile (solvent B) as mobile phase. The gradient (v/v) was generated keeping 90% of solvent A for 5 min, than decreasing to 65% in 15 min; and to 10% in 20 min and remaining at this concentration for 10 min. Before each injection the system was stabilised for 10 min with the initial A/B ratio (90:10, v/v). Chromatograms and spectra were elaborated with a ChromQuest V. 2.51 data system (ThermoQuest, Rodano, Milan, Italy).

2.7. Moisture content, total polyphenols and antioxidant activity assays

Moisture content was assessed by drying 1 g of sample for 4 h in a thermostatic oven at 105 ± 1 °C and weighing after it reached a constant weight. The total phenol content, antiradical (DPPH test) and total antioxidant (FRAP test) activities were measured through spectrophotometric determinations as described by Tuberoso et al. (2009).

3. Results and discussion

Honey-based product *abbamele* is produced from the honey heated up to 100 °C. Table 1 shows that final product vary significantly according to the preparation way. Moisture ranges from 15.3 to 27.7 g/100 g, with a variation of 55% and total sugar amount (determined by HPLC-RI) range from 55.2 g/100 g of the sample 3 to 75.3 g/100 g of sample 1. Sucrose was detected in the samples 1 and 5 and the ratio fructose/glucose is similar to that of honey showing a slight predominance of fructose. Differences among *abbamele* samples seem more connected with procedure of preparation, rather than with different types of honey used. The long thermal treatment involved in the production of *abbamele* caused very high content of 5-hydroxymethyl-furfural (HMF) in the samples. HPLC-DAD analysis showed the amount of HMF up to 4405.8 mg/kg in sample 5 (Table 1), values comparable with those found by (Spano et al. (2008)). The HMF values detected in *abbamele* ought to be compared with those of other heated food products, not the honey (Spano et al., 2008).

The two complementary isolation techniques used for the volatiles' isolation (HS-SPME and USE) followed by GC and GC–MS analyses allowed to obtain very representative chemical composition of *abbamele* more and less volatile compounds without the formation of artefacts. This approach is common among our research efforts for adequate fingerprinting of the honey volatiles in research of typical volatile marker compounds of unifloral botanical origin (Jerković, Marijanović, Kezić, & Gugić, 2009; Jerković, Tuberoso, Marijanović, Jelić, & Kasum, 2009). Some single compounds or groups of compounds have been reported by different researchers as indicative of the honey floral type, e.g. nonanol, nonanal, nonanoic acid and acetoin as being characteristic of eucalyptus honey (Perez, Sanchez-Brunete, Calvo, & Tadeo, 2002); acetophenone, 1-phenylethanol and 2-acetophenone being characteristic of chestnut honey (Guyot, Bouseta, Scheirman, & Collin, 1998).

Heat treatment in food is related to the transformations (mainly Maillard reactions) in flavour, aroma, taste and colour closely related with temperature, time, pH, the nature of reactants (i.e., the type of carbohydrates and amino acids or proteins in the honey), etc. (Martins, Jongen, & Van Boeckel, 2001). Heating the honey at temperatures as low as 50 °C leads to the formation of new volatile

Table 2
Compounds isolated from *abbamele* samples 1–7 by ultrasonic solvent extraction (USE) and headspace solid-phase microextraction (HS-SPME) analysed by GC and GC–MS.

No.	Compound	RI	1			2			3			4			5			6			7		
			A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Area percentage (%)																							
1	3-Methylbutanal*	>900	0.1	–	–	0.1	–	–	–	–	–	–	–	–	0.1	–	–	0.9	–	–	–	–	–
2	2-Methylbutanal*	>900	0.4	–	–	0.3	–	–	–	–	–	–	–	–	0.4	–	–	0.7	–	–	–	–	–
3	2,5-Dimethylfuran*	>900	–	–	–	–	–	–	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–
4	Dimethyl disulphide*	>900	0.1	–	–	0.1	–	–	–	–	–	–	–	–	0.3	–	–	0.7	–	–	0.1	–	–
5	Octane	>900	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–	0.4	–	–	–	–	–
6	2-Methyl-3-oxo-tetrahydrofuran*	>900	–	–	–	0.1	–	–	0.5	–	–	–	–	–	0.2	–	–	0.1	–	–	–	–	–
7	3-Methylbutanoic acid (Isovaleric acid)	>900	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.4	–	–	–	–	–
8	2-Furancarboxaldehyde (Furfural)	>900	2.9	–	–	3.6	–	–	13.6	–	–	1.3	–	–	7.3	–	–	7.3	–	–	20.4	–	–
9	2-Furanmethanol	>900	–	0.1	–	0.3	0.1	0.1	0.6	0.1	–	0.1	0.1	–	0.1	0.4	–	0.2	0.1	–	0.1	0.1	–
10	4-Methyloctane*	>900	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
11	Ethylbenzene	>900	–	0.1	–	–	0.1	–	–	0.1	–	–	–	–	–	–	–	–	0.1	–	–	–	–
12	1,4-Dimethylbenzene**	>900	–	0.4	–	–	0.3	–	–	0.6	–	–	0.2	–	–	0.2	–	–	0.7	–	–	0.2	–
13	Ethenylbenzene*	>900	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14	1,3-Dimethylbenzene**	>900	–	0.1	–	–	0.1	–	–	0.1	–	–	–	–	–	–	–	–	0.1	–	–	–	–
15	Phenylacetylene*	>900	0.5	–	–	–	–	–	4.5	–	–	–	–	–	–	–	–	1	–	–	–	–	–
16	Nonane	900	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–	0.3	–	–	–	–	–
17	2-Buthoxyethanol*	907	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
18	1-(2-Furanyl)-ethanone	914	0.8	0.2	0.1	1	0.1	–	4.2	0.1	–	0.3	–	0.1	1	0.1	–	2	0.2	0.1	3.7	0.4	0.1
19	α-Pinene	939	–	–	–	0.2	–	–	–	–	–	0.4	–	–	–	–	–	–	–	–	–	–	–
20	2-Methylpropyl-2-methyl butanoate*	945	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.6	–	–	–	–	–
21	5-Methyl-2-furfural	965	1.1	0.1	–	1.3	0.1	–	6.1	0.1	–	0.5	–	0.1	2.6	0.2	–	1.5	–	0.1	7.5	0.4	0.1
22	Hexanoic acid	974	–	–	–	1.7	0.3	–	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–
23	Dimethyl trisulphide*	975	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.3	–	–	–	–	–
24	β-Pinene	981	–	–	–	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–	–	–	–
25	β-Myrcene	992	–	–	–	0.8	–	–	–	–	–	1.1	–	–	–	–	–	–	–	–	–	–	–
26	α-Phellandrene	1007	–	–	–	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–	–	–	–
27	δ-3-Carene	1014	–	–	–	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–	–	–	–
28	α-Terpinene	1020	0.3	–	–	–	–	–	–	–	–	0.6	–	–	–	–	–	–	–	–	–	–	–
29	*p*-Cymene	1028	0.4	–	–	–	–	–	–	–	–	0.5	–	–	0.2	–	–	3.2	–	–	0.1	–	–
30	2-Hydroxy-3-methyl-cyclopent-2-en-1-one*	1031	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
31	Limonene	1032	4.1	–	–	70.8	0.4	0.5	0.4	–	–	76	0.2	0.5	0.5	–	–	1.6	–	–	15.8	–	–
32	Benzyl alcohol	1037	–	–	–	–	–	–	0.6	–	–	–	–	–	0.6	0.1	–	0.7	–	–	0.1	–	–
33	Phenylacetaldehyde	1048	4.6	0.4	0.2	0.3	–	–	3.2	0.1	–	0.2	–	0.1	0.7	–	–	2.9	–	0.1	0.8	–	–
34	*trans*-β-Ocimene*	1051	–	–	–	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–	–	–	–
35	4,7-Dimethylundecane**	1061	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
36	γ-Terpinene	1062	0.2	–	–	–	–	–	–	–	–	9.5	–	–	–	–	–	–	–	–	–	–	–
37	2-Acetylpyrrole*	1063	–	–	–	0.4	0.1	–	–	0.5	–	–	0.6	–	–	–	–	–	–	–	–	–	–
38	*trans*-Linalool oxide	1076	1.3	0.1	–	0.3	–	–	0.8	–	–	–	–	–	1	–	–	5.1	–	–	0.2	–	–
39	2-Furancarboxylic acid	1080	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
40	Methyl 2-furoate*	1084	0.8	–	–	0.8	–	–	2.5	–	–	0.4	–	–	2	–	–	3.1	–	–	3.3	–	–
41	1-(2-Furanyl)-2-hydroxyethanone*	1087	–	2.5	3	–	0.3	0.4	–	–	4.2	–	–	3.1	–	2.4	1.4	–	2.3	5.7	–	3.7	0.8
42	α-Terpinolene	1090	–	–	–	–	–	–	–	–	–	1.2	–	–	–	–	–	–	–	–	–	–	–
43	*p*-Cymenene	1092	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7.8	–	–	–	–	–
44	Fenchone	1093	9.3	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.7	–	–
45	Linalool	1101	0.3	–	–	1.7	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–	–	–	–
46	Nonanal	1105	0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3.7	–	–	–	–	–
47	2-Methylbenzofuran	1110	–	–	–	–	–	–	–	2.4	2.3	–	–	–	0.3	–	–	0.4	–	0.1	1.5	–	–
48	2-Phenylethanol	1116	–	–	–	–	–	–	51.5	–	–	0.4	–	–	–	–	–	0.7	–	–	–	–	–
49	3-Hydroxy-2-methyl-4*H*-Pyran-4-one*	1119	–	0.1	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
50	2-Ethylhexanoic acid*	1121	0.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
51	3,5,5-Trimethyl-cyclohex-2-en-1-one (Isophorone)	1124	2.5	0.1	–	–	–	–	–	–	–	–	–	–	30.9	0.4	–	1.5	–	–	–	–	–
52	2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-Pyran-4-one*	1144	–	0.6	0.5	0.2	0.3	–	–	0.1	–	–	0.2	0.4	0.8	1.7	0.9	1	1	2.6	–	0.5	0.6

53	3,5,5-Trimethylcyclohex-3-ene-1,4-dione (4-Ketoisophorone)	1147	–	–	–	–	–	–	–	–	–	–	–	–	1.9	–	–	2.5	–	–	–	–	–
54	Camphor	1148	0.4	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
55	2-Hydroxy-3,5,5-trimethyl-cyclohex-2-en-1-one* (2-Hydroxyisophorone)	1151	–	–	–	–	–	–	–	–	–	–	–	–	1.5	–	–	–	–	–	–	–	–
56	Benzoic acid	1162	–	0.1	–	–	0.2	–	–	0.3	–	–	0.4	0.6	–	0.1	–	–	–	0.1	–	0.2	–
57	Octanoic acid	1174	–	–	–	0.7	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
58	Terpinen-4-ol	1180	0.2	–	–	0.3	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–
59	α-Terpineol	1192	1.2	–	–	–	–	–	–	–	–	0.9	–	–	–	–	–	–	–	–	15.4	–	–
60	Methyl salicylate	1195	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.7	–	–	–	–	–
61	Methyl chavicol (Estragole)	1199	52.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
62	1-Methoxy-4-(prop-2-enyl)-benzene*	1202	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
63	Decanal	1207	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–
64	α-Ionene*	1213	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.9	–	–	–	–	–
65	3-Phenylfuran*	1223	0.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.8	–	–	–	–	–
66	5-Hydroxymethylfurfural	1230	2.9	27.7	51.3	3.1	17.3	49.6	1.9	6.9	21	0.5	19.3	76.5	16.9	57.6	77.3	6.1	17	65.3	19.5	78.7	91.2
67	4-(1-Methylethyl)-benzaldehyde (Cuminal)	1243	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–
68	Carvotanacetone*	1250	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.8	–	–	–	–	–
69	Nonanoic acid	1273	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3.4	–	–	–	–	–
70	2,4,6-Trimethyphenol**	1274	–	–	–	–	–	–	–	–	–	–	–	–	3.7	–	–	–	–	–	–	–	–
71	2-Phenylbut-2-enal*	1275	0.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
72	Phellandral*	1276	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4.9	–	–	–	–	–
73	Phenylacetic acid	1269	–	0.1	–	–	0.3	–	–	0.4	–	–	–	–	–	–	–	–	–	–	–	0.6	–
74	Anethole	1289	0.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
75	4-(1-Methylethyl)-benzenethanol* (*p*-Cymen-7-ol)	1292	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.8	–	–	–	–	–
76	Carvacrol	1294	–	–	–	0.7	–	–	1.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–
77	5-Acetyl-2-furanmethanol*	1301	–	–	0.1	–	0.1	–	–	–	–	–	–	0.2	–	–	–	–	–	0.1	–	–	–
78	2-Methoxy-benzene-1,4-diol*	1302	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
79	Thymol	1303	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	0.5	–	–	–	–	–
80	3-Methoxyacetophenone*	1306	–	–	–	–	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–
81	3-Phenylprop-2-en-1-ol** (Cinnamyl alcohol)	1313	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–
82	δ-Elemene*	1314	–	–	–	1.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
83	3,4,5-Trimethylphenol**	1317	0.4	0.1	–	–	–	–	–	–	–	–	–	–	16.1	1.2	0.5	3.5	–	0.1	–	–	–
84	1,2-Dihydro-1,1,6-trimethyl-naphtalene*	1354	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.9	–	–	–	–	–
85	Decanoic acid	1370	0.1	–	–	0.6	0.6	0.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
86	α-Copaene*	1377	–	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–
87	Ethyl decanoate (Ethyl caprate)	1397	–	–	–	–	–	–	0.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–
88	4-Hydroxybenzyl alcohol	1426	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–
89	3-Phenylprop-2-enoic acid (Cinnamic acid)	1434	–	–	–	–	0.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
90	Methyl-4-hydroxybenzoate	1471	–	0.1	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–
91	5-Hydroxydec-2-enoicacid lactone*	1478	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.6	–	–	–	–	–
92	5-Methyl-2-phenylhex-2-enal*	1490	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.8	–	–	–	–	–
93	δ-Selinene*	1491	–	–	–	0.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
94	Pentadecane	1500	–	0.1	–	–	0.1	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–
95	4-Methyl-2,6-bis-(1,1-dimethylethyl)-phenol	1514	–	0.4	–	–	0.4	0.6	–	0.3	–	–	0.2	0.1	–	0.4	–	0.4	0.6	0.1	–	0.3	–
96	4-Hydroxybenzoic acid	1522	–	0.2	–	–	1.1	–	–	0.8	–	–	15.5	1	–	3.3	0.1	–	–	–	–	–	–
97	Methyl 4-hydroxy-3-methoxybenzoate (Methyl vanillate)	1524	–	0.2	0.1	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–
98	4-Phenylbut-3-enoic acid*	1531	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
99	4-Hydroxy-3-methoxy-benzoic acid (Vanillic acid)	1566	–	–	–	–	–	–	–	–	–	–	0.4	–	–	–	–	–	–	–	–	–	–
100	Dodecanoic acid	1578	–	–	–	–	0.2	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
101	4-Keto-α-ionone*	1655	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.8	1.8	–	–	–	–
102	α-Ionol*	1656	–	–	–	–	–	–	–	–	–	–	0.4	0.2	–	0.3	0.2	–	0.7	0.3	–	–	–
103	Menthofuran	1662	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3	–	–	–	0.5	–	–	–
104	3-Hydroxy-4-phenyl-2(5*H*)-furanone*	1697	–	0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
105	2,4,5-Trimethoxy-3-methylphenol**	1728	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
106	Methyl 4-hydroxy-3-methoxyphenylacetic acid* (Methyl homovanillate)	1752	–	0.1	–	–	–	–	–	0.2	–	–	1.5	0.8	–	0.8	0.1	–	0.9	0.6	–	–	–
107	Tetradecanoic acid	1768	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

(*continued on next page*)

Table 2 (*continued*)

No.	Compound	RI	1			2			3			4			5			6			7		
			A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
108	Methyl 3,5-dimethoxy-4-hydroxybenzoate (Methyl syringate)	1774	–	49.2	29.9	–	4	1.4	–	29.3	35.4	–	2.5	0.4	–	1.3	1.1	–	1.5	0.8	–	0.2	–
109	4-Hydroxy-3,5,6-trimethyl-4-(3-oxobut-1-enyl)-cyclohex-2-en-1-one*	1795	–	0.9	0.6	–	–	–	–	–	–	–	0.9	1.1	–	7.2	6.1	–	6.7	6	–	–	–
110	3-(4-Hydroxyphenyl)-prop-2-enoic acid* (4-Coumaric acid)	1796	–	–	–	–	2.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
111	1H-Indene*	1834	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
112	3-(4-Hydroxy-3-methoxyphenyl)-prop-2-enoic acid (Ferulic acid)	1867	–	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–
113	Diisobutyl phthalate	1869	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.2	–	–	–
114	Hexadecan-1-ol	1882	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	0.2	–	0.1	0.2	–	–	–
115	Nonadecane	1900	–	–	–	–	0.1	–	–	0.1	–	–	–	–	–	1.6	–	–	43.8	0.5	–	–	–
116	1,2,3-Trimethoxy-5-(prop-2-enyl)-benzene* (Elemicin)	1923	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
117	Methyl hexadecanoate (Methyl palimitate)	1934	–	–	–	–	0.3	0.7	–	0.2	0.6	–	–	0.2	–	–	–	–	–	0.5	–	–	–
118	1-Phenoxypropan-2-ol*	1957	–	–	–	–	–	–	–	–	–	–	0.8	–	–	–	1	–	–	–	–	–	–
119	Hexadecanoic acid (Palmitic acid)	1963	–	0.2	0.3	–	17.9	22.3	–	15.8	11.4	–	11.6	3.4	–	2	0.3	–	–	–	–	0.5	0.6
120	Eicosane	2000	–	–	–	–	–	–	–	–	–	–	–	–	–	2.9	–	–	13.5	9.9	–	–	–
121	Ethyl hexadecanoate (Ethyl palmitate)	2002	–	–	–	–	0.9	–	–	0.7	–	–	–	–	–	–	–	–	–	–	–	–	–
122	(*Z*)-octadec-9-enoic acid (Oleic acid)	2147	–	–	–	–	–	–	–	0.5	–	–	–	–	–	–	–	–	–	–	–	–	–
123	2-Ethyl-dibenzothiophene*	2061	–	0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
124	(*Z*)-octadec-9-en-1-ol	2060	–	–	0.4	–	–	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–	–
125	Octadecan-1-ol	2084	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–	–	–
126	Methyl (*Z*,*Z*)-octadeca-9,12-dienoate (Methyl linoleate)	2101	–	–	–	–	1.4	0.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
127	Methyl (*Z*,*Z*,*Z*)-octadeca-9,12,15-trienoate (Methyl linolenate)	2109	–	–	–	–	1.1	1.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
128	Heneicosane	2100	–	0.8	5.1	–	–	–	–	6.4	1.2	–	5.3	0.5	–	0.2	0.1	–	2.2	0.3	–	0.3	–
129	(*Z*,*Z*,*Z*)-octadeca-9,12,15-trien-1-ol*	2175	–	–	–	–	–	–	–	–	–	–	6.9	–	–	–	–	–	–	–	–	–	–
130	(*Z*,*Z*)-Octadeca-9,12-dienoic acid (Linolenic acid)	2178	–	–	–	–	31.5	5.2	–	27	11.1	–	8.4	2.7	–	1	0.8	–	1.1	–	–	–	0.5
131	Octadecanoic acid	2181	–	–	–	–	–	–	–	–	–	–	1.5	2.8	–	–	–	–	–	–	–	–	–
132	Ethyl (*Z*,*Z*,*Z*)-octadeca-9,12,5-trienoate (Ethyl linolenate)	2197	–	–	–	–	9.1	6.5	–	1.8	1.2	–	–	–	–	–	–	–	–	–	–	–	–
133	Bis(2-ethylhexyl) phtalate*	2276	–	0.1	0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
134	Tetracosane	2400	–	1	–	–	2.5	2.4	–	3.1	2	–	11.6	1.5	–	0.5	–	–	1	0.1	–	0.2	–
	Total identified (%)		90.2	88.0	91.8	92.2	94.6	92.4	92.4	98.7	90.4	95.6	88.6	96.5	89.6	86.2	90.1	88.6	95.4	94.4	90.2	86.3	93.9

1–7-number of *abbamele* sample; **A**-HS-SPME; **B**-USE with the mixture of pentane and diethyl ether (1:2 v/v); **C**-USE with dichloromethane; RI-retention indices on HP-5MS column.

* tentatively identified (no reference compound was available).

** correct isomer not identified.

compounds (generation of artefacts) and the GC peak areas of many compounds varied significantly as a result of different heating conditions (Visser, Allen, & Shaw, 1988) either due to the oxidation or through Maillard reactions (Alissandrakis, Tarantilis, Harizanis, & Polissiou, 2005). The effect of heating on HMF content of honey is greatly influenced by the honey botanical origin (Fallico, Zappala, Arena, & Verzera, 2004).

3.1. Headspace solid-phase microextraction (HS-SPME)

Seventy-six compounds were detected with the headspace solid-phase microextraction (Table 2, column A). Thermally derived furan derivatives were abundant: 2-furancarboxaldehyde (1.3–13.6%), 5-methyl-2-furancarboxaldehyde (0.5–6.1%), methyl furancarboxylate (0.4–3.1%), 5-hydrohymethylfurfural (0.5–16.9%), dihydro-2-methyl-3(2*H*)-furanone (0.0–0.5%) and 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (0.0–1.0%). Their headspace percentages are not reliable, due to high water solubility and low volatility. On the other hand lower aliphatic aldehydes and acids (3-methylbutanal (0.0–0.9%), 2-methylbutanal (0.0–0.7%) and 3-methylbutanoic acid (0.0–0.4%)) were only identified by HS-SPME. They are also indicators of heat treatment and oxidation reactions. Thermally derived compounds are *abbamele* characteristic, but not indicating the honey type used in the production.

Terpenes were abundant in *abbamele* headspace, particularly limonene (0.5–76.0%) that probably originated from citrus rinds addition during production process. Limonene predominated in the samples 4 (76%) and 2 (70.8%) and it can be pointed out as specific aromatic headspace compound of *abbamele*, not found in such high concentrations in different honeys. Therefore, limonene headspace percentage is a key characteristic for detection of added citrus rinds during traditional and industrial processing of *abbamele*. Other monoterpenes were also present (not in all the samples) with minor percentages, such as α- and β-pinene (0.0–0.4%), β-myrcene (0.0–1.1%), α-phellandrene (0.0–0.2%), δ-3-carene (0.0–0.2%), α-terpinene (0.0–0.6%), *p*-cymene (0.0–0.5%), *trans*-β-ocimene (0.0–0.2%), γ-terpinene (0.0–9.5%), α-terpinolene (0.0–1.2%) and others, Table 2. Very few sesquiterpenes such as α-copaene (0.0–0.3%) and γ-selinene (0.0–0.8%) were found. Other honey ubiquitous terpenes such as linalool (0.0–1.7%) and *trans*-linalool oxide (0.0–5.1%) were also present. It is interesting to observe that samples 3 and 5 show just a very little amount of limonene and no other terpenes were detected. It can be supposed that in such samples only small amount of citrus peels was added. HS-SPME enabled detection of terpenes that are moderately visible in USE extracts probably due to higher volatility and consequently more abundance in the headspace.

Ubiquitous honey volatile compounds–benzene derivatives were found such as benzyl alcohol (0.0–0.7%), phenylacetaldehyde (0.2–4.6%) or 2-phenylethanol (0.0–51.5%). Highest content of 2-phenyethanol was found in sample 3, but it is not a specific marker since it has been reported in most honeys from a wide range of floral sources. However, high percentage of 2-phenyethanol was characteristic for several European honeys such as *Amorpha fruticosa* honey (Jerković, Marijanović et al., 2009; Jerković, Tuberoso et al., 2009) or *Calluna vulgaris* honey (Guyot, Scheirman, & Collin, 1999).

High content (30.9%) of isophorone in sample 6 (Fig. 1A) followed by minor percentage of 4-ketoisophorone (1.9%), 2-hydroxyisophorone (0.0–1.5%) and norisoprenoides in some samples (Table 2) could be connected with Sardinian strawberry-tree honey (*Arbutus unedo* L.). In fact, analysis of volatile (Bianchi, Cereri, & Musci, 2005; de la Fuente, Sanz, Martínez-Castro, Sanz, & Ruiz-Matute, 2007) and semi-volatile fractions (Dalla Serra et al., 1999) of strawberry-tree honey showed the presence of norisoprenoid compounds that are useful to characterise such a honey and were proposed as specific markers.

3.2. Ultrasonic solvent extraction (USE)

Four solvents with different polarities were tested on representative *abbamele* sample to determine the most suitable ones for the extraction of all samples with respect to the overall number of extracted compounds (data not shown). From this preliminary research, two different solvents (mixture of diethyl ether and pentane 1:2 v/v (solvent B) and pure dichloromethane (solvent C) were selected for USE in order to obtain more complete *abbamele* profile of more and less polar volatile and semi-volatile compounds. 5-Hydroxymethylfurfural was the predominant component in all USE extracts with the following distribution: solvent B (6.9–78.7%), solvent C (21.0–91.2%). Other furan and pyran derivatives were also present in USE extracts with solvent A such as 2-furanmethanol (0.1–0.4%), 5-methyl-2-furfural (0.1–0.2%), 3-hydroxy-2-methyl-4*H*-pyran-4-one (0.0–0.1%) or 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (0.1–1.7%). Several of these compounds were present in USE extracts with solvent C, Table 2. In general, the percentages of furan and pyran derivatives obtained by USE can be considered more reliable in comparison with HS-SPME due to higher polarity and less volatility.

Higher aliphatic acids (such as (*Z*)-octadec-9-enoic acid, octadecanoic acid and linolenic acid), alcohols (such as (*Z*)-octadec-9-en-1-ol and octadecan-1-ol) and esters (such as methyl hexadecanoate, ethyl hexadecanoate, methyl linoleate, ethyl linolenate and methyl linoleate) as well as higher hydrocarbons (such as tetracosane, heneicosane, eicosane and nonadecane) were abundant in the samples 2 and 3 probably originated from beewax (Jerković, Marijanović, Ljubičić, & Gugić, 2010) during *abbamele* traditional processing from honeycombs.

The most important marker of honey botanical origin found in USE extracts was methyl syringate (0.5–49.2%) with the highest percentages in the samples 1 and 3 (Table 2). Such high amounts of methyl syringate (Fig. 1B) can suggest that asphodel honey contributed in samples 1 and 3 productions. In fact, it is reported (Tuberoso et al., 2009) that Sardinian *Asphodelus microcarpus* honey is so far the honey with the highest amount of methyl syringate.

Very few terpenes in distinction from HS-SPME were identified and limonene was identified only in the samples 1 and 3 (Table 2). Isophorones were not found as in HS-SPME, but oxygenated norisoprenoides (such as 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-cyclohex-2-en-1-one) were identified in the samples 4, 5 and 6.

3.3. HPLC-DAD

Besides HMF, the used chromatographic method allowed to detect the specific markers of unifloral Sardinian honeys. Fig. 2 reports the HPLC-DAD fingerprinting of samples 1 and 5. Methyl syringate, marker of asphodel honey (Tuberoso et al., 2009), was found in the samples 1 and 3 (150.4 and 120.1 mg/kg, respectively). Homogentisic acid (85.2 mg/kg), unedone (112.9 mg/kg), *trans*,*trans*-abscisic acid (125.0 mg/kg) and *cis*,*trans*-abscisic acid (127.1 mg/kg), markers of strawberry-tree honey (Tuberoso et al., 2010), were detected only in the sample 5. These results confirm the information gathered from the volatile analyses: the samples 1 and 3 were prepared with high amount of asphodel honey, while Sardinian straw-berry tree honey was used only in *abbamele* 5. Heat treatment during *abbamele* production seems that it did not greatly influence the markers presence, although significant formation of thermal artefacts occurred.

3.4. Antioxidant activity and total phenol content

A last aspect that deserves attention is the antioxidant activities of *abbamele*. Total antioxidant activity measured with the FRAP assay ranged from 13.3 to 71.2 mmol Fe^{2+}/kg, while antiradical activity

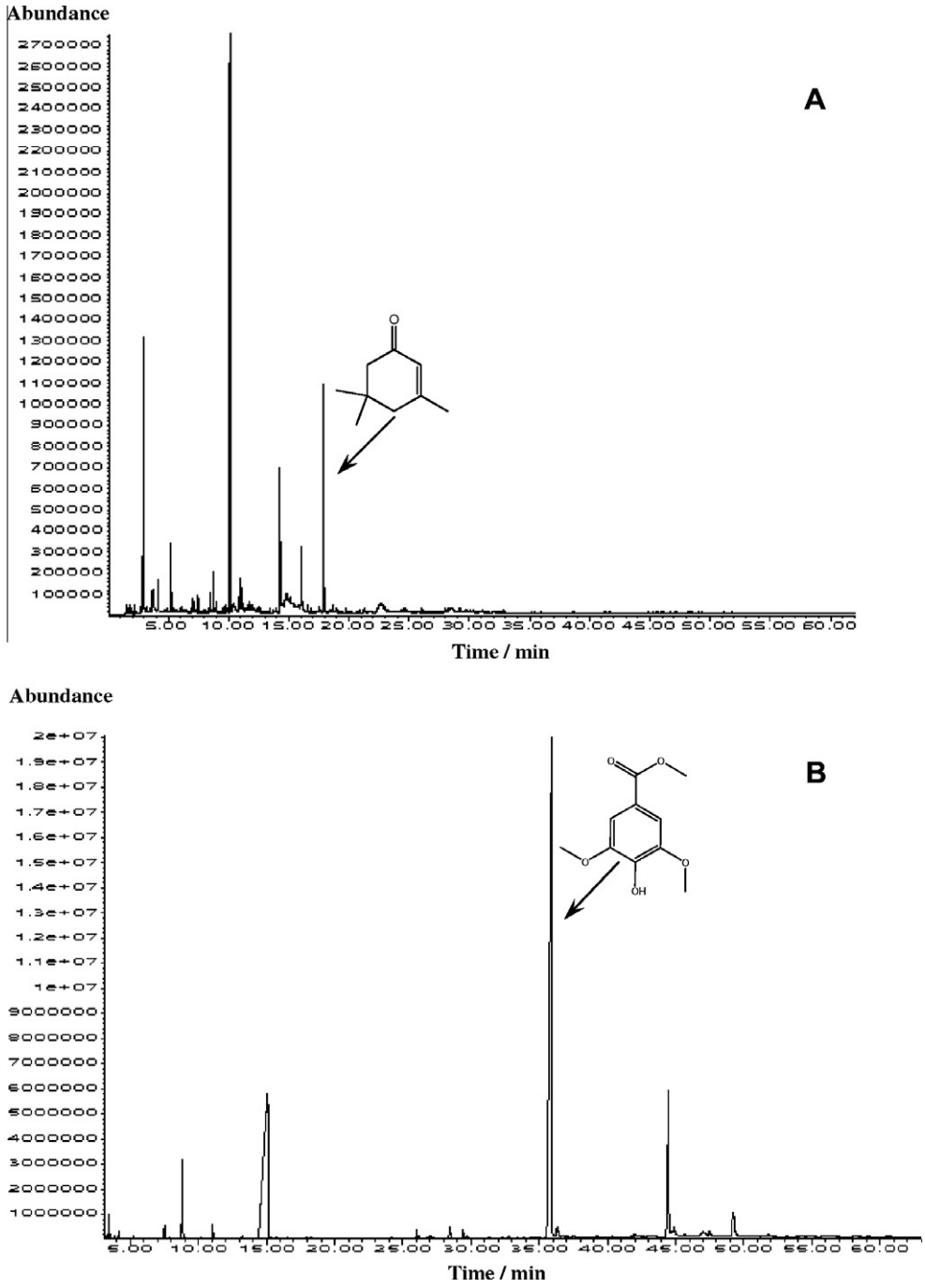

Fig. 1. Representative CG-MS chromatograms of *abbamele* samples 5 (**A**) and 1 (**B**) on HP-5MS column. Chromatogram (**A**) shows the marker compound isophorone obtained by HS-SPME, while chromatogram (**B**) shows the marker compound methyl syringate obtained by ultrasonic extraction with the mixture pentane diethyl ether (1:2 v/v).

measured with the DPPH assay ranged from 3.8 to 23.3 mmol TEAC/kg. Total phenolic amount ranged from 1297.8 to 4469.5 mg GAE /kg and it is linearly correlated with antioxidant and antiradical activities ($R^2_{\text{total phenols/FRAP}} = 0.9109$ and $R^2_{\text{total phenols/DPPH}} = 0.9395$). Values were very high if compared to those published for the honeys, although a direct comparison is very hard due to different types of antioxidant assay and way of quantification (Alvarez-Suarez, Tulipani, Romandini, Vidal, & Battino, 2009). However, dark and honeydew honeys that are known to have the highest levels of total phenolic compounds, usually do not exceed the level of 1250 mg GAE/kg (Al et al., 2009; Ferreira, Aires, Barreira, & Estevinho, 2009). FRAP values for honeys rich in phenolic compounds, such as chestnut, *Satureja hortensis* and honeydew honeys, ranged between 3.7 and 4.4 mmol Fe^{2+}/kg (Pichichero, Canuti, & Canini, 2009). Also comparison with other foodstuff is very interesting because total phenol amount is similar or even higher than products such as red wines (Heinonen, Lehtonen, & Hopia, 1998), fruits and vegetables (Brat et al., 2006). Total phenolic values found in the samples of *abbamele* are higher than those found by Spano et al. (2008), but differences can be due to different sample preparations and data expression. Nevertheless, evaluation of these data needs some considerations. As HPLC-DAD analysis did not show high amount of single phenolic compounds in *abbamele* samples, purification of *abbamele* samples on SPE Oasis HLB column according to Michalkiewicz, Biesaga, & Pyrzynska (2008) was performed. In this way it was expected to separate phenolic compounds from other

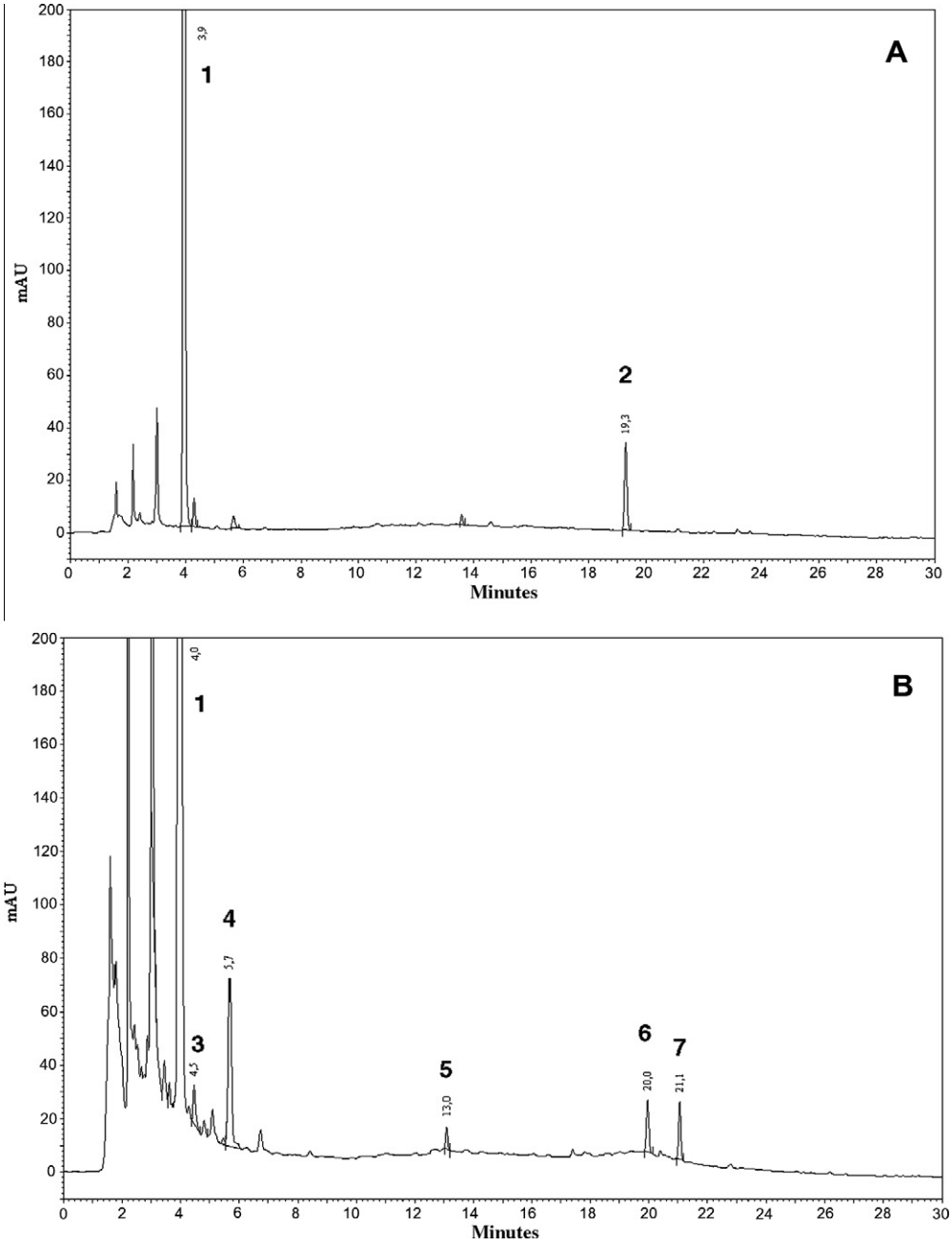

Fig. 2. Representative HPLC-DAD chromatograms of *abbamele* samples 1 (**A**) and 5 (**B**) at 280 nm. Chromatographic conditions are described in the text. 1) 5-(hydroxymethyl) furfural (HMF); 2) methyl syringate; 3) homogentisic acid; 4) furfural; 5) unedone; 6) *trans*,*trans*-abscisic acid; 7) *cis*,*trans*-abscisic acid.

eventually interfering compounds. Results showed that total phenol amounts in these extracts were not statistically different from the ones reported in Table 1 (data not shown). Interestingly, solutions containing extracted phenolic compounds were more or less brown, suggesting that the products of the Maillard reaction were not separated. However, because of the strong correlation between total polyphenols content and *abbamele* antioxidant activity found in this research, the total phenol amount is an interesting aspect, even affected from the contribution of Maillard reaction products. It is known that antioxidant activity of the honey is greatly influenced by its botanical origin (Frankel et al., 1998; Schramm et al., 2003), as well as heat treatment (Antony et al., 2000; Turkmen, Sari, Poyrazoglu, & Velioglu, 2006). Antioxidants are formed at several stages during the Maillard reaction, including degradation of Amadori compounds to amino reductones or reductones and the formation of polymers with antioxidant activity (Bailey & Um, 1992). Heterocyclic compounds, such as furan and pyran derivatives, proved to inhibit oxidation (Osada & Shibamoto, 2006; Yong-Xin, Li, Qian, Kim, & Kim, 2009). These products differ in molecular size and chemical structure with a common single antioxidative functional group, though the presence of entirely different antioxidants with different modes of action cannot be excluded (Lingnert, Eriksson, & Waller, 1983).

4. Conclusion

Seven *abbamele* samples, from different industrial and traditional producers, were obtained to study the relationship between

the samples' volatile aroma composition, honey marker compounds and the production procedures as well to determine their antioxidant activity. The investigation of the volatile fraction of *abbamele* with different extraction methods allowed obtaining different information about the production technology of this traditional product. The presence of typical markers of Sardinian unifloral honeys represents a powerful tool to connect *abbamele* with the territory of production. Terpenes are the key element to determine adding of citrus fruit. Total phenol amount and antioxidant activities showed to be very interesting because they are much higher than those of honeys and comparable with that of well-known products such as red wines and vegetables. The approach used in this study can be a model for the investigation of high-quality traditional foodstuff, because the presence of specific markers connected with natural ingredients or technological processes can support products' traceability and help to distinguish the products clearly from other similar ones.

Acknowledgements

This research was supported by UKF project 25/08 (Evaluation of Unifloral Honeys – Chemical Fingerprinting and Nutritional Properties), Fondazione Banco di Sardegna (funding 436/2007. 0065) and PIP d.o.o., KONCEPT-MEDIA d.o.o. and GODAX-PRO d.o.o.

References

Al, M. L., Daniel, D., Moise, A., Bobis, O., Laslo, L., & Bogdanov, S. (2009). Physico-chemical and bioactive properties of different floral origin honeys from Romania. *Food Chemistry., 112*, 863–867.

Alissandrakis, E., Tarantilis, P. A., Harizanis, P. C., & Polissiou, M. (2005). Evaluation of four isolation techniques for honey aroma compounds. *Journal of the Science of Food and Agriculture, 85*, 91–97.

Alvarez-Suarez, J. M., Tulipani, S., Romandini, S., Vidal, A., & Battino, M. (2009). Methodological aspects about determination of phenolic compounds and *in vitro* evaluation of antioxidant capacity in the honey: A review. *Current Analytical Chemistry., 5*, 293–302.

Antony, S. M., Han, I. Y., Rieck, J. R., & Dawson, P. L. (2000). Antioxidative effect of maillard reaction products formed from honey at different reaction times. *Journal of Agricultural and Food Chemistry, 48*, 3985–3989.

Bailey, M. E., & Um, K. W. (1992). Maillard reaction products and lipid oxidation. In *Lipid Oxidation in Food. ACS symposium series* (pp. 122–130). Washington, DC: American Chemical Society.

Bianchi, F., Cereri, M., & Musci, M. (2005). Volatile norisoprenoids as markers of botanical origin of Sardinian strawberry-tree (*Arbutus unedo* L,) honey: Characterisation of aroma compounds by dynamic headspace extraction and gas chromatography–mass spectrometry. *Food Chemistry, 89*, 527–532.

Brat, P., Georgé, S., Bellamy, A., Du Chaffaut, L., Scalbert, A., Mennen, L., et al. (2006). Daily polyphenol intake in France from fruit and vegetables. *The Journal of Nutrition, 136*, 2368–2373.

Dalla Serra, A., Franco, M. A., Mattivi, F., Ramponi, M., Vacca, V., & Versini, G. (1999). Aroma characterization of Sardinian strawberry tree (*Arbutus unedo* L,) honey. *Italian Journal of Food Science, 11*, 47–56.

de la Fuente, E., Sanz, M. L., Martínez-Castro, I., Sanz, J., & Ruiz-Matute, A. I. (2007). Volatile and carbohydrate composition of rare unifloral honeys from Spain. *Food Chemistry, 105*, 84–93.

El-Sayed, A.M. (2007). The pherobase: *Database of insect pheromones and semiochemicals*. Available from: http://www.pherobase.com.

Fallico, B., Zappala, M., Arena, E., & Verzera, A. (2004). Effects of conditioning on HMF content in unifloral honeys. *Food Chemistry, 85*, 305–313.

Ferreira, I. C. F. R., Aires, E., Barreira, J. C. M., & Estevinho, L. M. (2009). Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry., 114*, 1438–1443.

Frankel, S., Robinson, G. E., & Berenbaum, M. R. (1998). Antioxidant capacity and correlated characteristics of 14 unifloral honeys. *Journal of Apicultural Research, 37*, 27–31.

Guyot, C., Bouseta, A., Scheirman, V., & Collin, S. (1998). Floral origin markers of chestnut and lime tree honeys. *Journal of Agricultural and Food Chemistry, 46*, 625–633.

Guyot, C., Scheirman, V., & Collin, S. (1999). Floral origin markers of heather honeys: *Calluna vulgaris* and *Erica arborea*. *Food Chemistry, 64*, 3–11.

Heinonen, I. M., Lehtonen, P. J., & Hopia, A. I. (1998). Antioxidant activity of berry and fruit wines and liquors. *Journal of Agricultural and Food Chemistry, 46*, 25–31.

Jerković, I., Marijanović, Z., Ljubičić, I., & Gugić, M. (2010). Contribution of the bees and combs to honey volatiles: Blank-trail probe for honey biodiversity chemical profiling. *Chemistry and biodiversity, 7*, 1217–1230.

Jerković, I., Marijanović, Z., Kezić, J., & Gugić, M. (2009). Headspace, volatile and semi-volatile organic compounds diversity and radical scavenging activity of ultrasonic solvent extracts from *Amorpha fruticosa* honey samples. *Molecules, 14*, 2717–2728.

Jerković, I., Tuberoso, C. I. G., Marijanović, Z., Jelić, M., & Kasum, A. (2009). Headspace, volatile and semi-volatile patterns of *Paliurus spina-christi* unifloral honey as markers of botanical origin. *Food Chemistry, 112*, 239–245.

Lingnert, H., Eriksson, C. E., & Waller, G. R. (1983). Characterization of antioxidative Maillard reaction products from histidine and glucose. In *The Maillard Reaction in Foods and Nutrition. ACS Symposium Series* (Vol. 215, pp. 335–345). Washington, DC: American Chemical Society.

Martins, S. I. F. S., Jongen, W. M. F., & Van Boeckel, M. A. J. S. (2001). A review of Maillard reactions in food and implications to kinetic modelling. *Trends in Food Science & Technology, 11*, 364–373.

Michalkiewicz, A., Biesaga, M., & Pyrzynska, K. (2008). Solid-phase extraction procedure for determination of phenolic acids and some flavonols in honey. *Journal of chromatography A, 1187*, 18–24.

Osada, Y., & Shibamoto, T. (2006). Antioxidative activity of volatile extracts from Maillard model systems. *Food Chemistry, 98*, 522–528.

Perez, R. A., Sanchez-Brunete, C., Calvo, R. M., & Tadeo, J. L. (2002). Analysis of volatiles from Spanish honeys by solid phase microextraction and gas chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry, 50*, 2633–2637.

Pichichero, E., Canuti, L., & Canini, A. (2009). Characterisation of the phenolic and flavonoid fractions and antioxidant power of Italian honeys of different botanical origin. *Journal of the Science of Food and Agriculture, 89*, 609–616.

Schramm, D. D., Karim, M., Schrader, H. R., Holt, R. R., Cardetti, M., & Keen, C. L. (2003). Honey with high levels of antioxidants can provide protection to healthy human subjects. *Journal of Agricultural and Food Chemistry, 51*, 1732–1735.

Spano, N., Ciulu, M., Floris, I., Panzanelli, A., Pilo, M. I., Piu, P. C., et al. (2008). Chemical characterization of a traditional honey-based Sardinian product: *Abbamele*. *Food Chemistry, 108*, 81–85.

Spiggia, S. (1997). *Le api nella tradizione popolare della Sardegna*. Sassari, Italy: Carlo Delfino Editore. pp. 43–44.

Tuberoso, C. I. G., Bifulco, E., Caboni, P., Cottiglia, F., Cabras, P., & Floris, I. (2010). Floral markers of strawberry-tree (*Arbutus unedo* L.) honey. *Journal of Agricultural and Food Chemistry, 58*, 384–389.

Tuberoso, C. I. G., Bifulco, E., Jerković, I., Caboni, P., Cabras, P., & Floris, I. (2009). Methyl syringate: A chemical marker of asphodel (*Asphodelus microcarpus* Salzm. Et Viv.) monofloral honey. *Journal of Agricultural and Food Chemistry, 57*, 3895–3900.

Turkmen, N., Sari, F., Poyrazoglu, E. S., & Velioglu, Y. S. (2006). Effects of prolonged heating on antioxidant activity and colour of honey. *Food Chemistry, 95*, 653–657.

Visser, F. R., Allen, J. M., & Shaw, G. J. (1988). The effect of heat on the volatile fraction from a unifloral honey. *Journal Apicultural Research, 27*, 175–181.

Yong-Xin, L., Li, Y., Qian, Z.-J., Kim, M.-M., & Kim, S.-K. (2009). *In vitro* antioxidant activity of 5-HMF isolated from marine red alga *Laurencia undulata* in free radical mediated oxidative systems. *Journal of Microbiology and Biotechnology, 19*, 1319–1327.