

GC-MS Fingerprints and Other Physico-chemical Characteristics of Rare Unifloral *Prunus cerasus* L. Honey

Piotr Marek Kuś^a, Igor Jerković^{b,*}, Carlo Ignazio Giovanni Tuberoso^c, Zvonimir Marijanović^d and Mladenka Šarolić^d

^aDepartment of Pharmacognosy, Wrocław Medical University, ul. Borowska 211a, 50-556, Wrocław, Poland

^bDepartment of Organic Chemistry, Faculty of Chemistry and Technology, University of Split, N. Tesle 10/V, 21000 Split, Croatia

^cDepartment of Life and Environmental Sciences, University of Cagliari, via Ospedale 72, 09124 Cagliari, Italy

^dDepartment of Food Technology, Marko Marulić Polytechnic in Knin, Petra Krešimira IV 30, 22300 Knin, Croatia

igor@ktf-split.hr

Received: February 24th, 2013; Accepted: March 13th, 2013

GC-MS fingerprints of unifloral sour cherry (*Prunus cerasus* L.) honey were investigated for the first time by GC-FID and GC-MS {after headspace solid phase microextraction (HS-SPME) and ultrasonic solvent extraction (USE)}. Additionally, other physico-chemical characteristics of the samples were determined (total phenolic content, antioxidant activity and CIE L*a*b*C*h chromatic coordinates). The principal volatile components of the honey headspace were lilac aldehydes (46.0; 50.6%) along with benzaldehyde (18.0; 19.4%). The dominant component of the dichloromethane USE extract was vomifoliol (39.6; 44.9%). The abundant identified compounds may only serve as non-specific markers of the honey's botanical origin since they also occur in other honey types. The honey contained low-moderate amount of polyphenols (209.0 - 309.5 mg GAE/kg) and exhibited moderate antioxidant activity (0.4 - 0.6 mmol TEAC/kg; 1.6 - 1.9 mmol Fe²⁺/kg).

Keywords: *Prunus cerasus* L. honey, GC-FID, GC-MS, Lilac aldehydes, Vomifoliol, Kynurenic acid.

Sour cherry (*Prunus cerasus* L.) is an allotetraploid species that is supposed to be the result of natural hybridization of ground cherry (*P. fruticosa* L.) and sweet cherry (*P. avium* L.). The sour cherry fruits contain significant levels of antioxidants, mainly polyphenols such as anthocyanins and other flavonoids, chlorogenic and caffeic acids, as well as the alkaloid, melatonin [1]. Volatile compounds were also found in the fruits, including aliphatic and aromatic hydrocarbons, carbonyls, alcohols and esters such as phenylacetaldehyde, benzaldehyde, benzyl alcohol, linalool, hexanal, (*E*)-hex-2-enal, (*2E,6Z*)-nona-2,6-dienal, eugenol and vanillin [2,3].

Sour cherry honey is a rare honey type characterized by the taste of bitter almonds [4]. Unifloral honey of this plant is possible to obtain only in large monocultural orchards, because, in the same period (April-May), many alternative nectar sources are available, among them other fruit trees such as sweet cherry, plum, pear and apple [5]. Sour cherry flower may secrete 0.2-9.0 mg of nectar with 12-65% sugar content; additionally, extra-floral nectaries on the petioles are present [4]. To our best knowledge, till now, the metabolomic composition of sour cherry honey has not been analyzed. However, the composition of flavonoids in "cherry blossom honey" [6] (undetermined species) was determined (galangin, kaempferol, quercetin and isorhamnetin). The goal of this study is to determine the composition of volatiles present in sour cherry honey by GC-MS and GC-FID analyses. Additionally, its physico-chemical parameters (phenolic content, antioxidant activity and CIE L*a*b*C*h chromatic coordinates) were determined. This paper presents a continuation of our previous research on *P. mahaleb* L. honey [7] in order to further explore the biodiversity of *Prunus* spp. honey types.

Table 1: Pollen composition of analyzed honey samples.

	Sample I	Sample II
Specific pollen (%)	<i>Prunus</i> spp. (65%)	<i>Prunus</i> spp. (51%)
Other pollen (%)	<i>Salix</i> spp. (9%)	<i>Salix</i> spp. (12%)
	<i>Castanea sativa</i> (6%)	<i>Taraxacum</i> type (11%)
	<i>Rhamnus</i> spp. (3%)	<i>Taraxacum officinale</i> (9%)
	<i>Acer</i> spp. (3%)	<i>Rubus</i> spp. (4%)
	<i>Fraxinus</i> spp.* (2%)	Brassicaceae (3%)
	<i>Brassica napus</i> (2%)	Asteraceae (2%)
	<i>Taraxacum officinale</i> (1%)	<i>Robinia pseudoacacia</i> (1%)
	Asteraceae (1%)	Lamiaceae (<i>Salvia</i> type) (1%)
	<i>Centaurea cyanus</i> (1%)	<i>Lotus</i> spp. (1%)
	Not identified (7%)	Not identified (5%)

*non nectariferous plants.

Two rare *P. cerasus* honey samples from Poland were investigated, selected on the basis of pollen analysis. The samples contained predominantly *Prunus* spp. pollen grains [65% (sample I) and 51% (sample II)] (Table 1).

Determined physico-chemical parameters of the honey samples are presented in Table 2. CIE L*a*b*C*h chromatic coordinates were determined by UV/VIS spectroscopy. Total phenols were found in the range 295.0 - 309.5 mg GAE/kg. The honey antioxidant capacity determined by DPPH and FRAP assays was 0.4 - 0.6 mmol TEAC/kg and 1.6 - 1.9 mmol Fe²⁺/kg, respectively. Phenolic content was similar to pale Polish honeys [8], e.g. acacia or rape (175.7 - 411.7 mg GAE/kg). The antioxidant activity by FRAP assay was very similar to that of Italian *Thymus* honey [9] (1.834 mmol Fe²⁺/kg); however, its phenolic content was lower (126.55 mg GAE/kg). Sour cherry honey scavenged DPPH radical with similar performance as Croatian *Salix* spp. honey (0.6 mmol TEAC/kg [10]).

Table 2: Physico-chemical characteristics of the honey samples.

Parameter	<i>Prunus cerasus</i>	
	Sample I	Sample II
Humidity [%] ^a	18.0 ± 0.1	21.0 ± 0.2
HMF [mg/kg]	<i>tr</i>	<i>tr</i>
Total Phenols [mg GAE/kg] ^a	309.5 ± 21.0	295.0 ± 12.6
DPPH [mmol TEAC/kg] ^a	0.6 ± 0.2	0.4 ± 0.1
FRAP [mmol Fe ²⁺ /kg] ^a	1.9 ± 0.2	1.6 ± 0.4
L ^{a,b}	73.9	66.9
a ^{a,c}	4.0	5.5
b ^{a,d}	58.4	58.0
C ^{a,e}	58.5	58.3
h ^f	86.0	84.5

^a expressed as average ± standard deviation (n=3). ^b Lightness, %. ^c Indicates red for positive value and green for negative value, %. ^d Indicates yellow for positive value and blue for negative value, %. ^e Chroma, %. ^f Hue, deg.

The samples were analyzed by means of GC-FID and GC-MS after headspace solid phase microextraction (HS-SPME) and ultrasonic solvent extraction (USE). Major constituents, out of 24 identified, in sour cherry honey headspace were found to be (sample I; sample II): isomers of lilac aldehyde (17.2%; 18.0%, 24.5%; 19.7%, 8.9%; 8.3%) and benzaldehyde (19.4%; 18.0%). Less abundant were phenylacetaldehyde (1.9%; 1.4%), thymol (3.4%; 0.7%) and eugenol (3.2%; 0.7%). Several C₁₃ and C₉ norisoprenoids were present such as *trans*-β-damascenone (1.2%; 1.9%), 3,4-dihydro-3-oxoedulan (1.8%; 1.6%) and α-isophorone (2.3%; 0.1%), as well as terpenes: pinocarvone (0.2%; 0.8%), hotrienol (1.3%; 1.5%), linalool (1.6%; 1.7%) and *cis*-/*trans*-linalool oxides (1.0%; 1.1%, 0.2%; 0.1%). Small amounts of nitriles were also detected: 2-methylpropanenitrile (0.4%; 0.7%), 2-methylbutanenitrile (0.0%; 1.8%) and phenylacetoneitrile (0.2%; 0.5%), Table 3.

Table 3: The volatiles of sour cherry honey samples isolated by HS-SPME.

No	Compound	RI ^a	Area [%]	
			Sample I	Sample II
1.	Dimethyl sulfide	<900	0.4	0.9
2.	Ethyl acetate	<900	0.0	0.8
3.	2-Methylpropanenitrile	<900	0.4	0.7
4.	2-Methylbutanenitrile [*]	<900	0.0	1.8
5.	Isoamyl alcohol	<900	0.0	1.2
6.	3-Methylpentanal	<900	0.0	0.6
7.	3-Methylpentan-1-ol	<900	0.0	3.4
8.	Benzaldehyde	970	19.4	18.0
9.	Phenylacetaldehyde	1053	1.9	1.4
10.	<i>trans</i> -Linalool oxide	1080	1.0	1.1
11.	<i>cis</i> -Linalool oxide	1095	0.2	0.1
12.	Linalool	1107	1.6	1.7
13.	Hotrienol	1112	1.3	1.5
14.	α-Isophorone	1130	2.3	0.1
15.	Phenylacetoneitrile	1147	0.2	0.5
16.	Lilac aldehyde ^b	1151	17.2	18.0
17.	Lilac aldehyde ^b	1159	24.5	19.7
18.	1-Ethenyl-4-methoxybenzene (<i>p</i> -Vinylanisole)	1161	0.2	1.7
19.	Pinocarvone	1168	0.2	0.8
20.	Lilac aldehyde ^b	1174	8.9	8.3
21.	Thymol	1311	3.4	0.7
22.	Eugenol	1365	3.2	0.7
23.	<i>trans</i> -β-Damascenone	1390	1.2	1.9
24.	3,4-Dihydro-3-oxoedulan	1488	1.8	1.6

^a RI: Retention indices determined relative to *n*-alkanes (C₉–C₂₅) on HP-5MS column. ^b Correct isomer not identified. ^{*} Tentatively identified.

Benzaldehyde and lilac aldehydes are often found in various honey types. The latter were reported to occur abundantly and to be characteristic compounds of New Zealand nodding thistle honey [11], Greek citrus honey [12] and Croatian *Prunus mahaleb* L. honey [7]. Benzaldehyde is known to be responsible for the characteristic smell of bitter almonds where it is generated as a product of amygdalin hydrolysis [13] and plants from the genus *Prunus* are known to commonly contain cyanogenic glycosides, such as amygdalin. Its aroma was described as “sweet, almond, marzipan”.

Table 4: The volatiles of sour cherry honey samples isolated by USE.

No	Compound	RI ^a	Area [%]	
			Sample I	Sample II
1.	1-Methoxy-2-propyl acetate [*]	<900	0.4	0.8
2.	Benzaldehyde	970	0.4	0.6
3.	Benzyl alcohol	1037	0.3	0.4
4.	2-Phenylethanol	1116	0.1	0.4
5.	Phenylacetoneitrile	1150	0.1	0.4
6.	Lilac aldehyde ^b	1151	0.3	0.4
7.	Lilac aldehyde ^b	1159	0.1	0.3
8.	Lilac aldehyde ^b	1173	0.1	0.1
9.	Terpendiol I	1191	0.1	0.4
10.	Benzoic acid	1181	0.0	0.5
11.	Phenylacetic acid	1269	0.4	1.5
12.	Trimethylphenol ^p	1317	0.1	0.4
13.	2,6,6-Trimethyl-4-oxo-cyclohex-2-en-1-carboxaldehyde [*]	1319	1.8	1.4
14.	3-Methoxyacetophenone	1327	1.4	0.9
15.	Hydroxylinalool	1367	0.4	1.1
16.	Vanillin	1397	0.5	0.6
17.	3-Hydroxy-β-damascenone	1617	0.6	1.0
18.	Isopropylpseudocumene [*]	1661	0.4	0.5
19.	6,7-Dehydro-7,8-dihydro-3-oxo-α-ionol	1720	1.9	1.8
20.	3-(<i>p</i> -Hydroxy- <i>m</i> -methoxyphenyl)-2-propenal (coniferaldehyde)	1741	1.7	1.1
21.	Pentadecan-1-ol	1772	0.8	0.3
22.	9-Hydroxymegastigma-4,6-dien-3-one [*]	1773	4.2	4.2
23.	Vomifoliol	1825	44.9	39.6
24.	Hexadecan-1-ol	1882	3.2	4.6
25.	Nonadecane	1900	2.5	3.5
26.	(<i>Z</i>)-Octadec-9-en-1-ol	2060	8.9	10.0
27.	Octadecan-1-ol	2082	3.3	3.8
28.	Heneicosane	2100	1.3	0.4
29.	Tricosane	2300	0.1	0.3
30.	Tetracosane	2400	0.9	0.9

^a RI: Retention indices determined relative to *n*-alkanes (C₉–C₂₅) on HP-5MS column. ^b Correct isomer not identified. ^{*} - tentatively identified.

Therefore, the contribution of benzaldehyde may explain the specific bitter almond taste of sour cherry honey. Lilac aldehydes possess bitter taste that can be expressed with descriptors such as “pleasant, sweet, fresh, flowery”, and additionally their odor thresholds are very low [11,12], which implies their impact on the overall aroma of sour cherry honey may be very significant. Nitrile compounds were previously reported in *Taraxacum* labeled honey, but their provenience from this genus is difficult to explain since these compounds are not present in *Taraxacum* flowers, so the contribution of nectars from Brassicaceae was proposed as the source [14].

The analysis of dichloromethane USE extractives revealed 30 compounds (Table 4). The most abundant were vomifoliol (44.9%; 39.6%), (*E*)/(*Z*)-3-oxo-*retro*-α-ionol (1.9%; 1.8%, 4.2%; 4.2%) and coniferaldehyde (1.7%; 1.1%). The extract contained smaller quantities of phenylacetic acid (0.4%; 1.5%), 3-hydroxy-β-damascenone (0.6%; 1.0%), hydroxylinalool (0.4%; 1.1%) and vanillin (0.5%; 0.6%). Vomifoliol, a C₁₅ norisoprenoid, has been previously found as the major compound of *Mentha* spp. and *Prunus mahaleb* honey [7, 15].

Comparison of HS-SMPE and USE chromatographic fingerprints reveals significant differences in the distribution of volatiles. Low-molecular compound dominated in the headspace while the extracts contained a majority of semi-volatile compounds dominated by vomifoliol. Only few compounds were common for the HS-SPME and USE analyses and, therefore, combined fingerprinting of the honey headspace and extract is crucial for reliable identification of the more and less volatile compounds characteristic of sour cherry honey.

The volatile profile of *P. cerasus* honey was similar to that of *P. mahaleb* honey. Both contained a significant percentage of benzaldehyde and lilac aldehydes in the headspace, as well as vomifoliol in USE extractives, indicating a common *Prunus* spp. honey pattern. The above mentioned compounds were more abundant in sour cherry honey, but this honey did not contain coumarin, although this was present in *P. mahaleb* honey, as well as in the plant bark, flowers, leaves and wood [7]. Coumarin was, however, reported in sour cherry leaves [16]. *P. mahaleb* honey, unlike that of *P. cerasus*, contained abundant α -isophorone, 4-ketoisophorone and 4-anisaldehyde. On the other hand, the latter contained, for example, (*E*)- and (*Z*)-oxo-*retro*- α -ionol and *trans*- β -damascenone that were not found in *P. mahaleb* honey.

The volatiles from sour cherry honey exhibit some similarities with those found previously in the fruits. Both honey and fruits [2] contained ethyl acetate, benzaldehyde, phenylacetaldehyde, benzyl alcohol, benzoic acid, linalool, eugenol and vanillin. Important contributors to the fruit aroma [2], hexanal, (*E*)-hex-2-enal and (2*E*,6*Z*)-nona-2,6-dienal, were not detected in the current study. On the other hand, the fruits did not contain either lilac aldehydes or vomifoliol.

Experimental

Reagents: Dichloromethane and anhydrous sodium sulfate were purchased from Kemika (Zagreb, Croatia). Dichloromethane was redistilled before use. Acetonitrile, methanol, phosphoric acid 85% (w/w) were purchased from Merck (Darmstadt, Germany). The standards: gallic acid, (\pm)- (2*Z*,4*E*)-abscisic acid, kynurenic acid, 5-(hydroxymethyl)furfural (HMF), ferrous sulfate, sodium carbonate, ferric chloride, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), (\pm)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (Milan, Italy). (\pm)- (2*E*,4*E*)-Abscisic acid was purchased from A. G. Scientific, Inc. (San Diego, CA, USA). Ultrapure water (18 m Ω) was obtained with a Milli-Q Advantage A10 System (Millipore, Milan, Italy).

Honey samples: Two sour cherry (*Prunus cerasus*) honey samples were collected by a professional beekeeper in a sour cherry orchard in Poland during the spring of 2012. The honey's botanical origin was confirmed by pollen analysis to select the most reliable samples. Microscopical examination was carried out on a Hundh 500 (D-Wetzlar) light microscope equipped with a digital camera (Motic m 1000) supported by an image analysis system (Motic images plus software) for the morphometry of pollen grains.

Headspace solid-phase microextraction (HS-SPME): The extraction of headspace volatiles was carried out using a manual SPME fiber with a layer of polydimethylsiloxane/divinylbenzene (PDMS/DVB) obtained from Supelco Co (Bellefonte, PA, USA). The isolation was carried out from honey/NaCl saturated water solution (5 mL, 1:1, v/v) in 15 mL glass vials with a PTFE/silicone septa and a stirring bar. Equilibration time was set at 60 min, followed by 40 min sampling time. The fiber was transferred to the injector where the compounds were desorbed for 6 min. HS-SPME, followed by GC-FID and GC-MS were carried out in duplicate for each honey sample.

Ultrasonic solvent extraction (USE): The honey extraction was performed with dichloromethane in indirect sonication mode using an ultrasound bath (Elmasonic Typ S 30 H, Germany) at a frequency of 37 kHz at 25 \pm 3°C, as described previously [7]. The extraction of each sample was performed with 3 fresh portions of

solvent and the extracts were mixed together and concentrated to 0.2 mL by distillation with a Kuderna-Danish concentrator. One μ L of the obtained extract was used for GC-FID and GC-MS analyses.

GC-MS/GC-FID analysis: GC-FID analyses were carried out on an Agilent gas chromatograph model 7890A (Agilent Technologies, Palo Alto, CA, USA) with a flame ionization detector and a HP-5MS (5%-phenyl methylpolysiloxane, Agilent J & W GC column) capillary column (30 m x 0.25 mm i.d. 0.25 μ m film thickness). The oven temperature was held at 70°C for 2 min, then increased to 200°C at a rate of 3°C/min and then held isothermal for 15 min. Helium at 1 mL/min was used as carrier gas. Injector temperature was maintained at 250°C and detector temperature at 300°C. The analyses of VOCs by GC-MS were carried out with Agilent gas chromatograph model 7890A fitted with a mass selective detector model 5975C (Agilent Technologies, Palo Alto, CA, USA). Mass spectra were recorded in the electron impact ionization mode at 70 eV; the mass range was scanned in the *m/z* 50-300 range and the ion source temperature was 280°C. The volatile compound separation was obtained using the same column and oven temperature program as for GC-FID. The isolated compounds were identified by comparison of their retention indices (relative to C₉-C₂₅ *n*-alkanes) with available authentic samples and literature [17], as well as by comparing their mass spectra with the Wiley 275 MS library (Wiley, New York, USA) and NIST98 (Gaithersburg, Germany) mass spectral databases. The percentage composition of the samples was calculated from the GC peak areas using the normalization method (without correction factors) as a mean of triplicate analyses.

CIE $L^*a^*b^*C^*h^*$ chromatic coordinates determination: The measurements of chromatic coordinates were performed using an UV/VIS spectrophotometer Varian series Cary 50 Scan (Varian, Leini, TO, Italy), and data were managed with Cary Win UV Colour Application V. 2.00 software. Transmittances in a wavelength interval between 380 and 780 nm were measured using a D65 illuminant with a 10° observation angle. The honey samples were analysed fluid and transparent without any dilution in 10 mm optical polystyrene cuvettes (Kartell 01937).

Total phenols: Total phenol content was determined spectrophotometrically using a modified Folin-Ciocalteu method [18]. One hundred μ L of diluted honey solution (1 : 5, w/v, in ultrapure water) was added to 0.5 mL of Folin-Ciocalteu reagent. After 5 min, 3 mL of 10% Na₂CO₃, w/v, was added, and the mixture shaken and brought with H₂O to a final volume of 10 mL. After a 90 min incubation period at room temperature, spectrophotometric readings were made in a 10-mm quartz cuvette at 725 nm with a Varian Cary 50 Scan spectrophotometer against a blank. The total polyphenols contents, expressed as mg/kg of gallic acid equivalents (GAE), were calculated using a calibration curve made of a freshly prepared gallic acid standard solutions (10 - 500 mg/L).

Antiradical activity (DPPH test): A spectrophotometric analysis using DPPH radical and comparison with Trolox activity was performed, as described previously [18]. Fifty μ L of diluted honey (1:5 w/v, in ultrapure water) was dissolved in 2 mL of DPPH solution (0.04 mmol/L in MeOH). A calibration curve of Trolox was prepared (0.05 - 1.0 mmol/L) and data were expressed as Trolox equivalent antioxidant capacity (TEAC mmol/kg). The absorbance was read with a Cary 50 Scan spectrophotometer at 517 nm using a 10 mm quartz cuvette.

Total antioxidant activity (FRAP test): The ferric complex TPTZ and Fe³⁺ (0.3123 g TPTZ, 0.5406 g FeCl₃·6H₂O in 100 mL acetate

buffer pH 3.6) was prepared. Twenty μL of diluted honey solution (1 : 5, w/v, in ultrapure water) was dissolved in 2 mL of ferric complex. The quantitative analysis was performed by the external standard method (FeSO_4 , 0.1–2 mmol), correlating the absorbance read at = 593 nm with the concentration. The obtained results were expressed as mmol/kg of Fe^{2+} .

Water content: The honey water content was determined using a portable refractometer (ATAGO Hand Refractometer Honey, Atago Co. Ltd., Tokyo, Japan).

Acknowledgments - We are grateful to the Ministry of Science, Education and Sports of the Republic of Croatia for financial support (grant No: 011-0982929-1329).

References

- [1] Wang H, Nair MG, Strasburg GM, Booren AM, Gray JI. (1999) Antioxidant polyphenols from tart cherries (*Prunus cerasus*). *Journal of Agricultural and Food Chemistry*, **47**, 840-844.
- [2] Schmid W, Grosch W. (1986) Identification of highly aromatic volatile flavour compounds from cherries (*Prunus cerasus* L.). *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A*, **182**, 407-412.
- [3] Poll L, Petersen MB, Nielsen GS. (2003) Influence of harvest year and harvest time on soluble solids, titrateable acid, anthocyanin content and aroma components in sour cherry (*Prunus cerasus* L. cv. 'Stevnsbaer'). *European Food Research and Technology*, **216**, 212-216.
- [4] Farkas Á, Zajác E. (2007) Nectar production for the Hungarian honey industry. *The European Journal of Plant Science and Biotechnology*, **1**, 125-151.
- [5] Kemp H. (1996) Flowering phenology in European countries apple, pear, plum and cherry. In *Proceedings of the 2nd International Workshop on Pollination. Acta Horticulturae 423*. Tromp J, Wertheim SJ, Keulemans J. (Eds.), Lueven, Belgium, 299-300.
- [6] Petrus K, Schwartz H, Sontag G. (2011) Analysis of flavonoids in honey by HPLC coupled with coulometric electrode array detection and electrospray ionization mass spectrometry. *Analytical and Bioanalytical Chemistry*, **400**, 2555-2563.
- [7] Jerković I., Marijanović Z, Malenica Staver M. (2011) Screening of natural organic volatiles from *Prunus mahaleb* L. honey: coumarin and vomifoliol as nonspecific biomarkers. *Molecules*, **16**, 2507-2518.
- [8] Wilczyńska A. (2010) Phenolic content and antioxidant activity of different types of Polish honey - a short report. *Polish Journal of Food and Nutrition Science*, **60**, 309-313.
- [9] 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.
- [10] Tuberoso CIG, Jerković I, Bifulco E, Marijanović Z. (2011) Biodiversity of *Salix* spp. honeydew and nectar honeys determined by RP-HPLC and evaluation of their antioxidant capacity. *Chemistry & Biodiversity*, **8**, 872-879.
- [11] Wilkins AL, Lu Y, Tan ST. (1993) Extractives from New Zealand honeys. 4. Linalool derivatives and other components from nodding thistle (*Carduus nutans*) honey. *Journal of Agricultural and Food Chemistry*, **41**, 873-878.
- [12] Alissandrakis E, Tarantilis PA, Harizanis PC, Polissiou M. (2007) Aroma investigation of unifloral Greek citrus honey using solid-phase microextraction coupled to gas chromatographic-mass spectrometric analysis. *Food Chemistry*, **100**, 396-404.
- [13] Sánchez-Pérez R, Howad W, Garcia-Mas J, Arús P, Martínez-Gómez P, Dicenta F. (2010) Molecular markers for kernel bitterness in almond. *Tree Genetics & Genomes*, **6**, 237-245.
- [14] Soria AC, Martínez-Castro I, de Lorenzo C, Sanz J. (2008) Occurrence of nitriles in *Taraxacum* labelled honeys. *Food Chemistry*, **107**, 439-443.
- [15] Jerković I, Hegić G, Marijanović Z, Bubalo D. (2010) Organic extractives from *Mentha* spp. honey and the bee-stomach: methyl syringate, vomifoliol, terpenediol I, hotrienol and other compounds. *Molecules*, **15**, 2911-2924.
- [16] Shcherbanovskii L. (1965) On the presence of coumarin derivatives in sour cherry and prune leaves. *Ukrainskii Biokhimičeskii Zhurnal*, **37**, 915-919.
- [17] El-Sayed AM. (2012) *The Pherobase: Database of Insect Pheromones and Semiochemicals*, <http://www.pherobase.com>
- [18] Tuberoso CIG, 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.