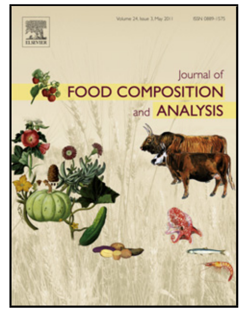
Accepted Manuscript



Title: Chemical markers for the authentication of unifloral *Salvia officinalis* L. honey

Author: Uroš M. Gašić Maja M. Natić Danijela M. Mišić Darija V. Lušić Dušanka M. Milojković-Opsenica Živoslav Lj. Tešić Dražen Lušić

PII:	S0889-1575(15)00174-X
DOI:	http://dx.doi.org/doi:10.1016/j.jfca.2015.08.008
Reference:	YJFCA 2619

To appear in:

Received date:	3-3-2015
Revised date:	20-6-2015
Accepted date:	5-8-2015

Please cite this article as: Gašić, U. M., Natić, M. M., Mišić, D. M., Lušić, D. V., Milojković-Opsenica, D. M., Tešić, Ž. Lj., and Lušić, D.,Chemical markers for the authentication of unifloral *Salvia officinalis* L. honey, *Journal of Food Composition and Analysis* (2015), http://dx.doi.org/10.1016/j.jfca.2015.08.008

Chemical markers for the authentication of unifloral *Salvia officinalis* L. honey

Uroš M. Gašić[a], Maja M. Natić[a], Danijela M. Mišić[b], Darija V. Lušić[cd], Dušanka M. Milojković-Opsenica[a], Živoslav Lj. Tešić[a], Dražen Lušić[c*]

[a]Faculty of Chemistry, University of Belgrade, P. O. Box 51, 11158 Belgrade, Serbia

[b]Institute for Biological Research "Siniša Stanković", University of Belgrade, Bulevar Despota Stefana 142 11060 Belgrade, Serbia

[c]Department of Environmental Health, Faculty of Medicine, University of Rijeka, Braće Branchetta 20, Rijeka HR-51000, Croatia

[d]Teaching Institute of Public Health of Primorsko-Goranska County, Krešimirova 52A, Rijeka HR-51000, Croatia

Highlights

► Chemical characterization of unifloral *Salvia officinalis* L. honey

► Determination of polyphenolics, carbohydrates and minerals

► Chemical markers for the authentication of *Salvia officinalis* honey

[*] Corresponding author: Dražen Lušić, Tel: ++ 385 51 334 530, Fax ++385 51 213 948;
E-address: drazen.lusic@medri.uniri.hr

ABSTRACT

The objective of the present study was to trace phytochemicals that characterize unifloral Common sage (*Salvia officinalis* L.) honey originating from the Croatian North Adriatic coast. The polyphenolic profiles and total phenolic contents (TPC), the compositions of minerals, sugars and sugar alcohols, and the radical scavenging activities (RSA) of 18 unifloral *S. officinalis* honey samples were investigated. The quantitative data on the targeted compounds (25 phenolic compounds, 14 carbohydrates and 25 minerals) together with the TPC and RSA data served as a pool of variables for multivariate analysis, which provided useful information for the accurate authentication of unifloral sage honey and its discrimination from other unifloral types of honey. The proposed markers, together with chemometrics, could further contribute, as a powerful tool, to the quality control of Croatian unifloral *S. officinalis* honey and thus, possibly certify its commercial value.

Keywords: Unifloral honey, *Salvia officinalis* L., Chemical markers, Polyphenolics, Sugars, Sugar alcohols, Minerals, Food analysis, Food composition

1. Introduction

Common sage (sometimes called Great sage or Dalmatian sage, Latin *Salvia officinalis* L.) is a circum-Mediterranean nectariferous botanical species common to the Eastern Adriatic and Ionian seas (Ricciardelli D'Albore and Galarini, 2000) with a habitat reaching south into northwest Greece (Karousou et al., 2000). This spontaneous perennial Mediterranean shrub (belonging to the family *Lamiaceae*), widespread in the Mediterranean part of Croatia, spontaneously grows on the hillsides of the North Croatian Littoral and Dalmatian islands as well in on the adjacent coastal belt (800–5000 m wide) and has significant beekeeping importance (Flora Croatica Database, 2012). The North–East part of the Adriatic Littoral (North Croatian Littoral) is especially characterized by an abundance of sage-dominated botanical communities. Actually, they sometimes cover areas of several square kilometers, representing practically the by far the most predominant plant of this poor, rocky terrain of the karst region (Šugar et al., 1983). Through the centennial tradition of beekeeping, this area has become well known for its famed unifloral *S. officinalis* honey that has seen widespread use in traditional medicine for the treatment of respiratory problems, as an antiseptic, *etc*. The potential health effects of this unifloral honey are usually ascribed to its phytochemical constituents, which mostly originate from *S. officinalis* nectar (Kenjerić et al., 2008).

The objective of the present study was to determine useful chemical markers for the authentication of unifloral *S. officinalis* honey, based on the analysis of the polyphenolic profiles, minerals, sugars and sugar alcohols in 18 honey samples originating from the Northnorth–-East east Adriatic region of Croatia. The pPhytochemical profiles of the studied honey samples were analyzed by high resolution LC/MS techniques. Quantification of major phenolic compounds was achieved using ultra ultra-high-performance liquid chromatography coupled with a diode array detector and a triple quadruple mass spectrometer (UHPLC DAD–MS/MS). In order to trace the phytochemicals that characterize sage honeys produced in the North Croatian Littoral, this work was focused on the identification of target compounds using ~~ultra~~ ultra-high-performance liquid chromatography coupled with hybrid mass spectrometry, which combined a Linear Trap Quadrupole and OrbiTrap mass analyzer (UHPLC–LTQ OrbiTrap MS). This technique has already proven itself to be reliable for the unambiguous detection of phenolic acids and their derivatives, as well as of the flavonoids aglycones and glycosides. The sugar content was determined using high-performance anion-exchange chromatography with pulsed amperomatric detection (HPAEC/PAD). The characterization of ~~Common~~ common sage unifloral honey was further supported by the evaluation of the mineral composition using inductively coupled plasma-atomic emission spectroscopy (ICP-OES) and melissopalynological analysis.

2. Experimental

2.1. Sage honey sampling and the authenticity of the samples

Representative honey sampling was performed directly at the filling facilities of the primary producer. After ~~sampling~~collection, samples were placed into ~~a~~ glass jars sealed with ~~the~~ metal lids and kept at ~~temperature of +~~4 ~~°C~~ to ~~+~~8 °C until analyzed. In order to attain confirmation of the botanical origin of the *S. officinalis* honeys, the samples were subjected to thorough melissopalynological and sensory assessment. Melissopalynological analysis, considered as an analytical tool essential for the verification of the botanical and geographical origin of a honey, was realized according to the method described by Loveaux et al. (1978) and further elaborated by Von der Ohe et al. (2004).

The extent to which a honey sample corresponds to a given plant source is determined from the frequencies of the pollen and honeydew elements in it. Since sage pollen is under-represented, and the percentage of sage pollen in the sediment is lower than the percentage of the corresponding nectar in the honey (Ricciardelli D'Albore and Galarini, 2000), the melissopalynological assessment was based on the expression of the pollen representativity within pollen frequency classes: "predominant pollen" (more than 45 % of the pollen grains); "secondary pollen" (16–45 %); "important minor pollen" (3–15 %); minor pollen" (less than 3 %), as well as on the presence of honeydew elements (Loveaux et al., 1978; Von der Ohe et al., 2004). Sensory assessment, as an equally important analytical mechanism for the determination of the unifloral character of a honey (Piana et al., 2004), comprehensive distinctive organoleptic features (visual, taste, odour, tactile) of the samples were determined taking into consideration the extent of their compliance with the organoleptic profile of unifloral sage honey (Lušić et al., 2007).

2.2. Reagents and standards

Acetonitrile and formic acid (both MS grade), methanol (HPLC grade), Folin~~–~~-Ciocalteu reagent, sodium carbonate, hydrogen peroxide, and hydrochloric and nitric acid were purchased from Merck (Darmstadt, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Sigma Aldrich (Steinheim, Germany). 2,2-Diphenyl-1-picrylhydrazyl·(DPPH·) was purchased from Fluka AG (Buchs, Switzerland). The Strata C18~~–~~-E (500 mg/3mL) SPE cartridges used for the extraction and concentration of samples were obtained from Phenomenex (~~ThermoFisher Scientific~~Torrance, CA). Ultra-pure water (Thermofisher TKA MicroPure water purification system, 0.055 µS/cm) was used to prepare the standard solutions and blanks. Syringe filters (13 mm, PTFE membrane 0.45 µm) were purchased from Supelco (Bellefonte, PA~~, USA~~).

cis, trans-Abscisic acid and polyphenolic standards were purchased from Fluka AG (Buchs, Switzerland). Sugar standards were purchased from Tokyo Chemical Industry (Zwijndrecht~~TCI, Europe~~, Belgium) and sugar alcohol standards were obtained from ~~Sigma~~ Sigma-Aldrich (Steinheim, Germany).

2.3. Preparation of standard solutions

A 1000 mg/L stock solution of a mixture of all phenolic standards and *cis, trans*-abscisic acid was prepared in methanol. Dilution of the stock solution with methanol yielded the working solutions of concentrations 0.025, 0.050, 0.100, 0.250, 0.500, 0.750, and 1.000 mg/L. Calibration curves were obtained by plotting the peak areas of the standards against their concentration~~Calibration curves were obtained by plotting the peak areas of the compounds identified relative to the peak area against the concentration of the standard solution~~. Calibration curves revealed good linearity, with ~~R~~r^2 values exceeding 0.99 (peak areas *vs*. concentration).

The evaluation of the carbohydrate content of the honey samples was obtained from calibration curves of pure compounds. The calibration was performed with standard solutions of sugars and sugar alcohols dissolved in ultrapure water. Each individual standard was dissolved in ultrapure water. Stock solutions with concentrations of 1000 mg/L were prepared and working solutions in the concentration ranges were as follows: for glucose and fructose from 10.0 to 100.0 mg/L; for sucrose from 1.0 to 10.0 mg/L; for isomaltose from 0.5 to 5.0 mg/L, while for all the other standards, the concentration range was from 0.1 to 1.0 mg/L. ~~Under these chromatographic conditions, the last compound was detected after approximately 25 min, and the analysis was ended at 30 min.~~

To analyze the mineral composition of honey, a multi-element plasma standard solution 4, Specpure, containing 1 g dm^{-3} of each element was utilized for reference purposes.

2.4. LC–MS/MS analysis

2.4.1. Preparation of sample extracts

The method previously described by Gasic et al. (2014) was used for extraction and isolation of phenolics from the honey samples. Prior to UHPLC–DAD MS/MS and UHPLC–MS/MS Orbitrap analysis, the extracts were filtered through a 0.~~45~~ 45-μm PTFE membrane filter.

2.4.2. UHPLC–MS/MS Orbitrap analysis of polyphenolic compounds

Separation of the compounds of interest ~~were~~ was performed using a liquid chromatography system that consisted of a quaternary Accela 600 pump and an Accela Autosampler, connected to a linear ion trap–orbitrap hybrid mass spectrometer (LTQ OrbiTrap XL) with a heated~~-~~ electrospray ionization probe, HESI-II (ThermoFisher Scientific, Bremen, Germany).

A Syncronis C18 column (100 × 2.1 mm, 1.7 μm particle size) from Thermo Fisher Scientific was used as the analytical column for separation. The mobile phase consisted of ~~(A)~~ water + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B)~~water + 0.1 % formic acid and (B) acetonitrile + 0.1 % formic acid~~. A linear gradient program at a flow rate of 0.300 mL/min was used: 0.0–1.0 min 5 % B, 1.0–9.9 min from 5 % to 95 % (B), 9.9–10 min from 95 % to 5 % (B), then 5 % (B) for 3 min. The injection volume was 5 μL (Gasic et al., 2014).

The mass spectrometer was operated in the negative ion mode. The HESI–source parameters were given previously (Gasic et al., 2014). Xcalibur software (version 2.1) was Xcalibur software 2.1 (Thermo Fisher, Bremen, Germany) was used for instrument control, data acquisition and data analysis. The phenolics were identified according to the corresponding spectral characteristics: mass spectra, accurate mass, characteristic fragmentation, and characteristic retention time. Full scan analysis was employed to detect the monoisotopic mass of unknown compounds, while the fragmentation pathway was obtained by MS/MS. This exact mass search method was based on high resolution MS analysis (Orbitrap), online database search (Patiny and Borel, 2013) and prediction of MS/MS fragmentation using Mass Frontier 6.0 software (Thermo Fisher Scientific).

2.4.3 UHPLC–DAD MS/MS analysis of polyphenolic compounds

The separation, determination, and quantification of the components in the sage honey samples were performed using a Dionex Ultimate 3000 UHPLC system equipped with a diode array detector (DAD) that was connected to TSQ Quantum Access Max triple-quadrupole mass spectrometer (ThermoFisher Scientific, Basel, Switzerland). The elution was performed at 40 °(C on a Syncronis C18 column. The mobile phase consisted of (A) water water + 0.1 % formic acid (A) and acetonitrile (B) + 0.1 % formic acid, and (B) acetonitrile, which were applied in the following gradient elution: 5 % B in the first 2.0 min, 2.0–12.0 min 5–95 % B, 12.0–12.2 min from 95 % to 5% B, and 5 % B until the 15th min. The flow rate was set to 0.4 mL min^{-1} 00.4 ml min^{-1} and the detection wavelengths to 254 – and 280 nm254 and 280 nm. The injection volume was 5 μlL.

A TSQ Quantum Access Max triple-quadrupole mass spectrometer equipped with an heated electrospray ionization (HESI) source was used with the vaporizer temperature kept at 200 °C, and the ion source settings as follows: spray voltage 5000 V, sheet gas (N_2) pressure

40 AU, ion sweep gas pressure 1 AU and auxiliary gas (N_2) pressure 8 AU, capillary temperature 300 °C, and skimmer offset 0 V (Natic et al., 2015). The mass spectrometry data were acquired in ~~the~~ negative ion mode, in the *m*/*z* range from 100 to 1000. Multiple mass spectrometric scanning modes, including full scanning (FS), and product ion scanning (PIS), were conducted for the qualitative analysis of the targeted compounds. The collision-induced fragmentation experiments were performed using argon as the collision gas, and the collision energy was varied depending on the compound (**Table S1**). The time-selected reaction monitoring (tSRM) experiments for quantitative analysis were performed using two MS^2 fragments for each compound that were previously defined as dominant in the PIS experiments (**Table S1**).

Xcalibur software 2.2 (Thermo Fisher, Bremen, Germany) ~~Xcalibur software (version 2.2)~~ was used for instrument control. The phenolics were identified by direct comparison with commercial standards. The total amounts of each compound were evaluated by calculation of the peak areas and are expressed as mg kg^{-1}.

2.5. Determination of TPC and RSA

The samples were prepared according to a previously described method (Gasic et al., 2014). Each honey sample (5 g) was mixed with ultrapure water in a 50~~-~~mL volumetric flask. The solution was then filtered through 0.45-µ~~l~~m PTFE membrane and analyzed for determination of TPC and RSA~~,~~. The amount of total phenolics was determined according to the Folin~~-~~-Ciocalteu method, while the radical-scavenging activity of honey extracts was measured using the DPPH· method (Gasic et al., 2014). The TPC and RSA values are expressed as milligram gallic acid equivalents (mg GAE) per~~equivalents (GAE) per~~ kilogram and micromoles of Trolox equivalents (µmol TE) per~~equivalents (TE) per~~ kg of honey sample, respectively.

2.6. HPAEC/PAD analysis of sugars and sugar alcohols

The honey samples were homogenized, weighed (between 0.2 and 0.3 g) and diluted 1000-fold with ultrapure water. The solutions were filtered and transferred to vials.

The sugar and sugar alcohol contents were determined by HPAEC/PAD.~~high performance anion exchange chromatography with pulse amperometric detection (HPAEC/PAD).~~ The honeys were analyzed on an ICS 3000 DP liquid chromatograph equipped with a quaternary gradient pump (Dionex, Sunnyvale, CA~~, USA~~). The carbohydrates were separated on a CarboPac®PA10 ~~pellicular~~ anion-exchange column (4 × 250 mm) at 30 °C. Each honey sample (25 µL) was injected with an ICS AS-DV 50 autosampler (Dionex~~, Sunnyvale, CA, USA~~). The carbohydrates were eluted with the flow rate set to 0.7 mL/min, using a gradient program constituted from 600 mM sodium hydroxide (eluent A), 500 mM sodium acetate (eluent B) and ultrapure water (eluent C). The gradient program was as follows: 0.0–20.0 min, 15 % A; 20.1–30.0 min, 20 % A; 0.0–5.0 min, 0 % B; 5.1–12.0 min, 2 % B; 12.1–20.0 min, 4 % B; 20.1–30.0 min, 20 % B, 0.0–5.0 min, 85 % C; 5.1–12.0 min, 83 % C; 12.1–20.0 min, 81 % C; 20.1–30.0 min, 60 % C. Under these chromatographic conditions, the last compound was detected after approximately 25 min, and the analysis was ended at 30 min. The total amounts of each sugar or sugar alcohol ~~was~~were evaluated according to the method ~~previously~~ described in section 2.3.

2.7. ICP−EOS analysis of minerals in honey samples

To analyze the mineral composition of honey, about 0.6–0.7 g of fresh honey sample ~~was~~ were treated with 7 mL of 65 % HNO_3 and 1 mL of 35 % H_2O_2 in polytetrafluoroethylene (PTFE) vessels. A microwave closed digestion system (ETHOS 1~~,~~;

Milestone, Bergamo, Italy) was used for the mineralization process. The final clear solution was made up to 50 mL with ultrapure water. A blank was prepared in the same way.

All mineral elements in the digested solutions were determined using an ICP-OES (iCAP 6500 Duo ICP, Thermo Scientific, UK) instrument. The results are expressed as mg of mineral~~metal~~ per kg of honey.

2.8. Statistical analysis

Data of all measurements performed in triplicate are expressed as the mean ± standard deviation (*SD*). Statistical analyses were performed using the Analysis ToolPak from ~~the~~ Microsoft Office Excel 2007 Professional. ~~Statistical analyses were performed with the program MS Excel (Microsoft Office 2007 Professional).~~ PCA was realized using the PLS~~-~~Tool Box software package for MATLAB 7.12.0 (Eigenvector Research, Inc., Wenatchee, WA~~, USA~~)~~MATLAB (Version 7.12.0)~~. All data were group-scaled prior to PCA. The singular value decomposition algorithm (SVD) and a 0.95 confidence level for Q and T^2 Hotelling limits for outliers were chosen.

3. Results and discussion

3.1. Verification of the sage honey samples

A great deal of attention was given to the authenticity of the Croatian Common sage honey samples, especially to their geographical and botanical origin (Persano Oddo and Bogdanov, 2004). Representative honey sampling was realized directly at primary producers' filling facilities, above all taking into ~~the~~ consideration two important criteria: A) that the honey sample extraction occurred ~~closely~~ soon after the sage flowering period (May) when sage flowers were the main bee source of nectar, and B) appropriate apiary locations for sample production. That is to say, particular beehive sites were selected for collection of *S. officinalis* honey samples in line with the field observations on the abundance of sage nectar. Furthermore, cartographic data concerning the areas of predominate *Salvia officinalis* L. growth were taken from the comprehensive ~~Vegetation~~ vegetation maps of Croatia (Šugar et al., 1983), confirming that the production beehives involved were situated deep~~ly inside~~ within the sage-dominated vegetation zones.

As a general rule, honey is considered unifloral if it ~~was~~ is produced mainly from one plant species, and if the pollen of that particular species predominates. However, the pollen grains of some flowers are under-represented (or over-represented) in unifloral honeys, *i.e.*, the percentage of pollen in the sediment is lower (or higher) than the percentage of the corresponding nectar in the honey (Persano Oddo and Bogdanov, 2004). Therefore, the pollen spectrum of other nectariferous and non-nectariferous botanical species should likewise be taken into the consideration, as well as the presence of honeydew elements (Persano Oddo and Bogdanov, 2004; Piazza and Persano Oddo, 2004). The unifloral character of all the sage honey samples in this study was confirmed by thorough melissopalynological and sensory evaluation (**Table 1**). When compared to the representation of other pollen sources in samples, under-representation of *S. officinalis* pollen grains was noted in almost all the studied honey samples, thereby confirming the natural hypopollenic features of sage (Ricciardelli D'Albore and Galarini, 2000; Flora Croatica Database, 2012). The ~~greatest~~ highest portion of the identified pollen in the sage unifloral honey originated from nectariferous species belonging to the families *Rhamnaceae*, *Sapindaceae* (genus *Acer*) and *Fagaceae* (genus *Castanea*). Pollen sources of non-nectariferous producing plants were mostly attributed to *Quercus* spp. (fam~~.~~ily *Fagaceae*) and species belonging to the families *Graminaceae* and *Plantaginaceae* (*Plantago* spp.), all sharing the flowering period of sage as well as their areal of distribution. This characteristic pollen profile and specific combination could be considered a valuable indicator of the geographical origin of the sage unifloral honey samples.

Sensory assessment, as an equally important analytical mechanism for the determination of the unifloral character of honey (Piana et al., 2004) revealed distinctive organoleptic features (visual, taste, odour, tactility) of the samples, taking into consideration the extent of their compliance with the particular organoleptic profile of unifloral sage honey (Lušić et al., 2007). Based on the results of the melissopalynological and sensory evaluations, all the honey samples in the present study were confirmed to be sage honeys.

3.2. Phenolic profile of Croatian sage honey samples

Although the composition of honey highly depends on the floral source used to collect the nectar, some other factors, including geographic origin, seasonal and environmental factors, bee variety, as well as processing technologies, may also affect the composition of the phenolic compounds in honey (Kaskonienė and Venskutonis, 2010). On the other hand, unifloral honeys have almost never been made from 100 % monofloral nectar, since the nectar from flowers of many various plants contributes to the production of every honey (Persano Oddo and Bogdanov, 2004). Therefore, it was important to analyze a large number of sage honey samples, in order to derive more general rules, and define which compounds and/or groups of compounds mostly characterize the phenolic and sugar profiles, and thus the uniqueness of this autochthonous honey. Phenolic compounds such as flavonoids (Kenjerić et al., 2008), carbohydrates (Primorac et al., 2011), and volatile compounds (Jerković et al., 2006), were previously suggested as possible markers for the determination of Common sage unifloral honey.

As it was previously reported, sage leaf extracts contain a wide range of phenolic compounds with the majority of the phenolic acids represented by caffeic acid derivatives and rosmarinic acid being the dominant oneSage leaf extracts, as hitherto reported, contain a wide range of phenolic compounds. The majority of the phenolic acids were found to be caffeic acid derivatives, with rosmarinic acid being the dominant one. The study of According to Generalić et al. (2011), identified rosmarinic, syringic, gallic, *p*-coumaric, caffeic, and *trans*-ferulic acid as the principal phenolic acids of Common sage extracts.the principal phenolic acids of Common sage extracts are rosmarinic, syringic, gallic, *p*-coumaric, caffeic, and *trans*-ferulic acid. The relative content of rosmarinic acid in the extracts ranged from 94.54-% to 98.38-%, depending on the phenophase, while the contents of other acids were significantly lower (Generalić et al., 2011; Generalić et al., 2012). Other studies also report the presence of vanillic acid, salvianolic acids K and I, and methyl rosmarinate in Common sage (Dragovic-Uzelac et al., 2012; Dent et al., 2013). Flavonoids of *S. officinalis* are mostly present as flavones (apigenin, luteolin and their corresponding 6-hydroxylated derivatives), flavone glucosides (6-hydroxyluteolin-7-glucoside, luteolin-7-glucuronide, luteolin-glucoside, luteolin-3′-glucuronide, apigenin-7-glucuronide and apigenin-7-glucoside), flavonols (mostly kaempferol and quercetin methyl ethers), and flavonol glucosides (quercetin-4′-glucoside, rutin), as reported by several authors (Generalić et al., 2011; Dragovic-Uzelac et al., 2012; Generalić et al., 2012). Stilbenes (*trans*-resveratrol, astringin, piceid) and catechins ((+)-catechin, (–)-epicatechin) are also present (Generalić et al., 2011; Generalić et al., 2012). *Salvia officinalis* L. is reported to contain also phenolic diterpenes, including carnosol and carnosic acid (Lamien-Meda et al., 2010). Some of the phenolic compounds previously determined as constituents of sage leaf extracts were also found in unifloral *S. officinalis* honeys from this region (Kenjerić et al., 2008), including phenolic acids (caffeic, rosmarinic, gallic, *p*-coumaric, and ferulic acid), and flavonoids (flavones apigenin and luteolin, and their corresponding glycosides; flavonols quercetin and kaempferol and their derivatives (quercetin hexoside and rutin); stilbenes (resveratrol); and catechins (catechin and epicatechin)). It should be borne in mind that previous studies concerning the composition of the phenolics in indigenous Croatian Common sage honey (Kenjerić et al., 2008) usually concentrated on targeted metabolomic analysis that included a limited number of compounds, and that there ~~is~~ are a lack of literature data concerning the complete polyphenolic profiles.

On the other hand, the present study gives insight into the profile of the phenolics of sage unifloral honey using the non-targeted metabol~~i~~omic approach, which resulted in the identification of a significant number of phenolic compounds (**Table 2**). In the absence of standards, the identification of flavonoid glycosides and other phenolics were based on the search for the $[M–H]^{-}$ deprotonated molecule and its fragmentation using UHPLC~~–~~-LTQ OrbiTrap MS/MS. The exact mass search and the study of the fragmentation pathways described in the literature enabled as much structural information as possible to be obtained. In this way, it was possible to ~~individuate~~ identify 61 compounds (**Table 2)**. The chromatograms of the investigated Common sage honey samples showed similar profiles. A selected base peak chromatogram of a representative sage honey extract (sample No. **SH2**) is shown in **Fig. S1**.

Hydroxycinnamic acids, such as caffeic, rosmarinic, ferulic, chlorogenic, and *p*-coumaric acid were detected in the sage honey samples analyzed in the present study. These phenolic acids constituted a significant share ~~to~~ of the total phenolics content of the sage honey samples. Generally, the presence of phenolic compounds in nectar is usually connected with their protective role against microbial infestations (Heil, 2011). However, high concentrations of these compounds could lead to ~~the~~ nectar's toxic effect, and have a negative influence on pollinators (Adler, 2000). Of the hydroxybenzoic acids, *p*-hydroxybenzoic acid, vanillic, gentisic, and protocatechuic acid were previously reported in sage (Zgórka and Głowniak, 2001), and confirmed in unifloral sage honey samples. All these phenolic acids are considered as potential markers for the authentication of sage unifloral honeys and were therefore included in the subsequent targeted quantitative analyses of the honey samples.

The majority of flavonoids in *S. officinalis* are flavones of apigenin and luteolin, and their corresponding 6-hydroxylated derivatives (hispidulin and cirsimaritin), as well as the dihydroflavone hesperetin (Brieskorn and Biechele, 1971; Cuvelier et al., 1996; Lu and Yeap Foo, 2002; Kontogianni et al., 2013), and all of these compounds were evidenced in the analyzed sage honey samples. Of the flavone~~,~~ glucosides, luteolin and apigenin glycosides are very common in analyzed sage honeys, and some of them were previously found in *S. officinalis* (Masterova et al., 1989; Wang et al., 1998; Lu and Yeap Foo, 2000). Interestingly, it is well known that the presence of 6-hydroxy- and 6-methoxy-flavone glycosides clearly differentiates section *Salvia*, which includes *S. officinalis*, from other sections belonging to the genus *Salvia* (Tomás-Barberán et al., 1988). Therefore, the presence of these compounds in honey might be one of the indicators that the honey in question is really of sage floral origin. Flavonols of sage are mostly those of kaempferol and quercetin methyl ethers (Lu and Yeap Foo, 2002), and nectar~~–~~-pollen derived flavonoids, such ~~are~~ as quercetin, kaempferol, and hesperetin, have been identified in samples of Common sage honey. Of the flavonoids previously identified in sage, stilbene resveratrol and catechins (catechin and epicatechin) were also confirmed in the sage honey samples (Generalić et al., 2011). The following derivatives of catechin and epicatechin were also recorded in the honey samples: gallocatechin, epigallocatechin, gallocatechin gallate, and epigallocatechin gallate.

The phenolic diterpenes carnosol and carnosic acid, although present in sage (Kontogianni et al., 2013), were not previously detected in unifloral sage honeys. In the present study, these compounds were identified in ~~the~~ all honey samples, but in trace amounts (**Table 2**).

3.3. Quantification of targeted phenolics in the honey samples

~~Solid~~ Solid-phase extraction (SPE) combined with ultra-~~high~~ high-performance liquid chromatography with a diode array detector (DAD) and a triple-quadrupole mass spectrometer was used to analyze the content of 25 targeted compounds in the *S. officinalis* honey samples. Three basic criteria for the selection of chemical markers from the group of phenolic compounds were applied: 1) putative sage nectar~~–~~-pollen derived compounds (phenolic acids and flavonoids); 2) propolis characteristic flavonoids and 3) abscisic acid.

Among the quantified compounds in Common sage honeys, some ~~of~~ phenolic acids, *i.e.*, *p*-coumaric, *p*-hydroxybenzoic, and ferulic acid, were present in the highest amounts. Interestingly, rosmarinic acid was present in relatively low amounts in the unifloral sage honeys analyzed in the present study (**Table 3**). It is well known that phenolic acids of sage are mostly based on caffeic acid building blocks (Lu and Yeap Foo, 2002), and that rosmarinic acid is the major phenolic compound in sage leaves. Possible reasons for this could be relatively low concentrations of this compound in the nectar. Gentisic acid was detected only in three samples (SH8, SH15, and SH18). Of the nectar~~–~~-pollen derived flavonoids quantified herein, quercetin, kaempferol, and hesperetin were abundant and present in significant amounts. Stilbene resveratrol was detected only in four of the sage honey samples (SH2, SH5, SH16, and SH17). Catechins were abundant in the analyzed honey samples, with gallocatechin gallate and epigallocatechin gallate being quantified as the dominant compounds from this group. The contents of catechin and epicatechin were low.

Pinocembrin, pinobanksin, pinostrobin, galangin, and chrysin are characteristic flavonoids of propolis, and were determined in most of the previously analyzed European honey samples (Tomás-Barberán et al., 2001; Kenjerić et al., 2008). The portion of propolis-derived compounds in the unifloral sage honeys analyzed in the present study was significant, but much less than in a previous study (Kenjerić et al., 2008), which reported a relatively high portion of galangin and chrysin (51.3 %) in the total identified flavonoids. The sage honey samples analyzed in the present study were characterized by the significant amounts of pinobaksin (0.21–2.35 mg/kg) and chrysin (0.06–1.98 mg/kg).

The plant stress hormone abscisic acid (Ab~~B~~A) is known to be present in floral nectars of some plants, and is transferred from the nectar to honey. This phytohormone is present in relatively high amounts in some European honeys (Tomás-Barberán et al., 2001; Truchado et al., 2008; Bertoncelj et al., 2011), including unifloral sage honey (Kenjerić et al., 2008), and was also confirmed in the present study. The presence of abscisic acid in high amounts (0.26–3.99 mg/kg) is not surprising, since natural rocky habitat of sage is characterized by periods of drought ~~seasons~~ during the summer, which results in stress-induced responses in the plants (Bertoncelj et al., 2011).

3.4. Antioxidant activity of Common sage honeys

Antioxidant capacity of *S. officinalis* honey samples was determined by the total phenolics content (TPC) and the radical scavenging activity (RSA). The results of these investigations are given in **Table 3**.

The Common sage honey samples were characterized with TPC values ranging between 20~~8.51~~9 ~~to~~ and 747~~.5~~4~~9~~ mg ~~of gallic acid equivalents (~~GAE~~)~~ per kg of honey. The average content of total phenolics was in a good agreement with the values given in the literature for sage honeys from the same region (Piljac-Žegarac et al., 2009).

The results of the determination of the RSA of sage honey samples ranged from 351~~.20~~ to 89~~4.~~8~~27~~5 micromoles of ~~Trolox equivalents~~TE per kg of sample. To determine the relationship between the content of polyphenols and antioxidant activities of *S. officinalis* honey samples, the correlation between the TPC and the RSA values was calculated. The RSA showed a statistically significant (r = 0.872; ~~P~~ p < 0.0001) and positive linear correlation with the TPC (*RSA* = 68.08 + 1.10 × *TPC*). A significant and positive linear relationship between the antioxidant activity and total phenolic content of sage honey samples indicated that phenolic compounds could be identified as the chemicals that predominately contributed to the antioxidant activity, which is in accordance with previous investigations ~~reported previously~~ (Piljac-Žegarac et al., 2009; Gasic et al., 2014).

3.5. Determination of the sugars and sugar alcohols

Fourteen different sugars and sugar alcohols were identified and quantified in the analyzed unifloral sage honey samples using ~~the~~ HPAEC/PAD ~~method~~. Quantification was performed with available standards. The reducing sugars, fructose and glucose, were found to be the major constituents of all the investigated samples (**Table 4**), which confirmed that all honey samples were genuine honeys. In all the analyzed honeys, the value of the glucose plus fructose amounts was around or higher than 60~~/~~ g per100 g, which is the value for all honey types required by the European and FAO (Codex Alimentarius) standards (FAO/WHO, 2001; The Council of the European Union, 2002). Another monosaccharide identified in the honeys in relatively low amounts was arabinose.

All the sage honey samples had a sucrose content lower than 5~~/~~ g per100 g, which is generally taken as the limit value for honeys allowed by European Union Honey Directive (The Council of the European Union, 2002). Apart from sucrose, the other identified disaccharides were trehalose, turanose, maltose and isomaltose. The trisaccharides maltotriose and isomaltotriose were also evidenced. From the group of polyols (sugar alcohols), erythritol, sorbitol, ~~galactytol~~galactitol, and glycerol were identified.

The ratio between some carbohydrates is another indicator that may be used to ascertain honey authenticity. Thus, the ratios of fructose/glucose, maltose/isomaltose, sucrose/turanose, and maltose/turanose, maltotriose/raffinose+erlose+melezitose were used for the authentication of some unifloral honeys, and all these studies were reviewed by Kaskonienė and Venskutonis (2010). The fructose/glucose (*FRU*/*GLU*) ratio in sage honeys, which was recommended for the evaluation of honey granulation because glucose is less ~~water-~~ water-soluble than fructose, varied from 1.31 in sample **SH1** to 4.42 in sample **SH~~8~~7**. One more characteristic of the unifloral sage honeys analyzed in the present study was the relatively low maltose/isomaltose (*MAL*/*iMAL*) ratio, which ranged from 0.9 (sample **SH11**) to 2.41 (**SH5**).

3.6. Determination of minerals in S. officinalis honeys

The concentrations of minerals quantified in the studied sage honey samples are presented in **Table 5**. The most abundant element in all samples was found to be potassium (content ranging from 592~~1.68~~ to 215~~1.35~~0 mg/kg), which agrees with other studies and indicates that K is the most common element in honey~~s~~ (Cantarelli et al., 2008), including unifloral sage honeys (Bilandžić et al., 2014). Phosphorus, sulfur, and calcium were the next most common elements, followed by magnesium and sodium. Among the micro-elements in decreasing amounts, B, Zn, Fe, Mn, Cu, Se, and Ni were found, while Co, Cr, Li, and V were found as trace elements. Therefore, the influence of botanical origin on the elemental composition of the unifloral sage honey was evident for both elements essential for plant growth (macronutrients), such as K, P, S, Ca, Mg and Na, and for micronutrients (trace elements), such as B, Mn, Zn, Fe, *etc*. The essential elements are present in plants in significantly higher amounts than the trace elements, and this observation was also true for the honey samples. On the other hand, the possibility that the mineral composition of honey samples also reflects the environmental and pedological conditions of the geographical locality cannot be excluded(Terrab et al., 2004). Toxic elements (Al, As, Cd, Pb, and Sb) in the tested samples were found in small amounts (allowable concentrations), which excludes the existence of environmental contamination of the honeys.

3.7. Pearson's correlation analysis

Pearson's correlation analysis was performed to evaluate the associations between variables in 18 sage unifloral honey samples (**Table S2**), in order to define some general rules characteristic for unifloral sage honey. Both positive and negative Pearson's correlations were observed between the contents of the different analyzed compounds in the unifloral sage honeys. However, statistically significant correlations were observed in some cases as can be seen from the Tables given in supplementary material (**Tables S2–S4**). High positive correlations were found between propolis-derived compounds. Namely, correlations between CaA and PNB, PNS, CHR, PNC, GLN were in the range from 0.691 to 0.886. Likewise, correlations among PNB, PNS, CHR, PNC, and GLN were also characterized with high positive coefficients (**Table S2**). Statistically significant correlations between CaA and HES ($r = 0.827$, ~~P~~ $p \leq 0.0005$), FeA and GeA ($r = 0.786$, ~~P~~ $p \leq 0.0005$), FeA and PrA ($r = 0.652$, ~~P~~ $p \leq 0.005$), C and EC ($r = 0.663$, ~~P~~ $p \leq 0.005$), and C and EGC ($r = 0.656$, ~~P~~ $p \leq 0.005$) could be considered as important characteristics of the analyzed sage honeys. It was also observed that AbA was well correlated with FeA and GeA, with $r = 0.890$ (~~P~~ $p \leq 0.000001$) and $r = 0.887$ (~~P~~ $p \leq 0.000001$), respectively. The observed correlations between the phenolic compounds in the analyzed honey samples probably reflected the situation in the sage nectar and/or pollen, which are the main sources of phenolics in honey.

Pearson's correlation analysis was also performed between 14 targeted carbohydrates in the unifloral sage honey samples (**Table S3**), whereby the highest positive correlation was observed between maltose and isomaltose ($r = 0.870$, ~~P~~ $p \leq 0.000005$), which could be considered as a unique characteristic of unifloral sage honey. Moreover, statistically significant correlations were found between ~~MALt and SUC, and MALt and TUR~~maltotriose and sucrose, and maltotriose and turanose (**Table S3**).

Regarding the mineral composition of the sage honeys, among all statistically significant correlations, the highest positive ones were between Ca and Mn with r = 0.858 (~~P~~ $p \leq$ 0.000005), and between Mg and P with r = 0.849 (~~P~~ $p \leq$ 0.000005). **Table S4** shows the Pearson's correlation analysis of the minerals.

3.8. Authentication of unifloral sage honey

In order to demonstrate the applicability of the present research for the authentication of unifloral sage honey, three types of available unifloral honeys of *Lamiaceae* species were introduced into the analysis as out-groups: mint (*Mentha* spp.) honey, winter savory (*Satureja montana* L.) honey, and thyme (*Thymus* spp.) honey. The quantitative data on TPC, RSA, targeted phenolics, sugars and minerals in thyme, mint and winter savory honeys are presented as Supplementary data (**Table S5**). Principal component analysis (PCA) was employed to analyze the quantitative data for TPC, RSA, 25 targeted phenolic compounds, 14 carbohydrates and 25 minerals in order to examine their relative variations within different honeys (sage, mint, thyme and winter savory honeys).

The combination of all the variables was informative enough to clearly discriminate sage honeys from the honeys of different floral origins. The results showed that the principal factorial 2-dimensional plane captured 32.18 % of the total variability (**Fig. 1**). The first principal component accounted for 17.58 % and the second for 15.60 % of the total variance. Clear differentiation of unifloral sage honey from unifloral thyme, mint and winter savory honeys along PC 1 was observed. The variables responsible for the differentiation of unifloral sage honey from the other studied honeys were identified using the loading plots (**Fig. 1B**). Sage honey samples were distinguished from the other studied honeys based on the significantly higher contents of ~~mineral~~ boron ~~B~~. Most of the samples of sage honeys were characterized with high K contents. Higher contents of TPC, ~~TUR~~ turanose and ~~KAE~~ kaempferol in the sage honeys compared to the thyme, mint and winter savory honeys further contributed to the separation (Fig. 1B). On the other hand, mint honey was characterized by larger contents of Mn, Ba, and ~~ChA~~ chlorogenic acid, when compared to the other samples. Only two unifloral sage samples (**SH2** and **SH8**) considerably deviated from the rest of the sage honey samples along PC2, due to higher contents of chrysin, pinocembrin, galangin~~CHR, PNC, GLN,~~ and ~~CaA~~caffeic acid, which were also characteristic for the **MH1** and **WSH2 samples**.

4. Conclusions

The study of sage (*Salvia officinalis* L.) honey samples showed ~~somewhat~~ interesting results related to their ~~peculiar~~ characteristic phenolic, sugar and mineral contents. Several identified compounds showed significant potential for the characterization of this particular honey ~~intrinsic for~~typical of the Adriatic Littoral of Croatia, especially its northern area. The data suggest clear differentiation of unifloral sage honey from the other unifloral honeys by using groups of chemical markers (phenolic compounds, carbohydrates and minerals). Among all studied unifloral honeys of *Lamiaceae* species, higher contents of boron and potassium, as well as turanose and kaempferol could be identified as authentication markers of unifloral sage honey. In addition, the application of multivariate statistical analysis ~~to~~ for the authentication and classification ~~was~~ proved to be an important complementary tool for a more reliable identification and quality control method of honey.

Acknowledgement

This work was performed within the framework of the Research Projects Nos. 172017 and 173024, supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia and specialized funds of the Department of Environmental Health, Faculty of Medicine, University of Rijeka.

References

Adler, L.S., (2000). The ecological significance of toxic nectar. Oikos 91(3), 409-420.

Bertoncelj, J., Polak, T., Kropf, U., Korošec, M., Golob, T., (2011). LC-DAD-ESI/MS analysis of flavonoids and abscisic acid with chemometric approach for the classification of Slovenian honey. Food Chemistry 127(1), 296-302.

Bilandžić, N., Gačić, M., Đokić, M., Sedak, M., Šipušić, Đ.I., Končurat, A., Gajger, I.T., (2014). Major and trace elements levels in multifloral and unifloral honeys in Croatia. Journal of Food Composition and Analysis 33(2), 132-138.

Brieskorn, C.H., Biechele, W., (1971). Flavones from Salvia officinalis. Compounds of Salvia officinalis. Archiv der Pharmazie (Weinheim) 304, 557–561.

Cantarelli, M.A., Pellerano, R.G., Marchevsky, E.J., Camiña, J.M., (2008). Quality of honey from Argentina: study of chemical composition and trace elements. The Journal of the Argentine Chemical Society 96, 33-41.

Cuvelier, M.-E., Richard, H., Berset, C., (1996). Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. Journal of the American Oil Chemists' Society 73(5), 645-652.

Dent, M., Dragovic-Uzelac, V., Penić, M., Brncic, M., Tomislav, B., Levaj, B., (2013). The Effect of Extraction Solvents, Temperature and Time on the Composition and Mass Fraction of Polyphenols in Dalmatian Wild Sage (Salvia officinalis L.) Extracts. Food Technology & Biotechnology 51(1), 84–91.

Dragovic-Uzelac, V., Elez Garofulić, I., Jukić, M., Penić, M., Dent, M., (2012). The Influence of Microwave-Assisted Extraction on the Isolation of Sage (Salvia officinalis L.) Polyphenols. Food Technology & Biotechnology 50(3), 377–383.

FAO/WHO, (2001). Codex standards for honey, in: Codex Alimentarius Commission (Ed.), *2nd revision*. Food and Agricolture Organisation of the United Nations, World Health Organisation, Rome, pp. 1-8.

Flora Croatica Database, (2012). Flora Croatica Database. Vascular Plants, Taxonomy & Bibliography of Croatian Flora, 2004 ed. Department of Botany, Faculty of science , FER-ZPR , University of Zagreb.

Gasic, U., Keckes, S., Dabic, D., Trifkovic, J., Milojkovic-Opsenica, D., Natic, M., Tesic, Z., (2014). Phenolic profile and antioxidant activity of Serbian polyfloral honeys. Food Chemistry 145, 599-607.

Generalić, I., Skroza, D., Ljubenkov, I., Katalinić, A., Burčul, F., Katalinić, V., (2011). Influence of the phenophase on the phenolic profile and antioxidant properties of Dalmatian sage. Food Chemistry 127(2), 427-433.

Generalić, I., Skroza, D., Šurjak, J., Mozina, S.S., Ljubenkov, I., Katalinić, A., Simat, V., Katalinić, V., (2012). Seasonal variations of phenolic compounds and biological properties in sage (Salvia officinalis L.). Chemistry & Biodiversity 9(2), 441-457.

Heil, M., (2011). Nectar: generation, regulation and ecological functions. Trends in Plant Science 16(4), 191-200.

Jerković, I., Mastelić, J., Marijanović, Z., (2006). A Variety of Volatile Compounds as Markers in Unifloral Honey from Dalmatian Sage (*Salvia officinalis* L.). Chemistry & Biodiversity 3, 1307-1310.

Karousou, R., Hanlidou, E., Kokkini, S., (2000). The sage plant of Greece: distribution and intraspecific variation, in: Kintzios, S.E. (Ed.), *Sage the genus Salvia*. Harwood Academic Publishers, Amsterdam, pp. 27–46.

Kaskonienė, V., Venskutonis, P.R., (2010). Floral Markers in Honey of Various Botanical and Geographic Origins: A Review. Comprehensive Reviews in Food Science and Food Safety 9(6), 620-634.

Kenjerić, D., Mandić, M., Primorac, L., Čačić, F., (2008). Flavonoid pattern of sage (*Salvia officinalis* L.) unifloral honey. Food Chemistry 110, 187-192.

Kontogianni, V.G., Tomic, G., Nikolic, I., Nerantzaki, A.A., Sayyad, N., Stosic-Grujicic, S., Stojanovic, I., Gerothanassis, I.P., Tzakos, A.G., (2013). Phytochemical profile of Rosmarinus officinalis and Salvia officinalis extracts and correlation to their antioxidant and anti-proliferative activity. Food Chemistry 136(1), 120-129.

Lamien-Meda, A., Nell, M., Lohwasser, U., Borner, A., Franz, C., Novak, J., (2010). Investigation of antioxidant and rosmarinic acid variation in the sage collection of the genebank in Gatersleben. Journal of Agricultural and Food Chemistry 58(6), 3813-3819.

Loveaux, J., Maurizio, A., Vorwohl, G., (1978). Methods of Melissopalynology by International Commission for bee Botany or IUBS. Bee World 59(4), 139-157.

Lu, Y., Yeap Foo, L., (2000). Flavonoid and phenolic glycosides from Salvia officinalis. Phytochemistry 55(3), 263-267.

Lu, Y., Yeap Foo, L., (2002). Polyphenolics of Salvia—a review. Phytochemistry 59(2), 117-140.

Lušić, D., Koprivnjak, O., Ćurić, D., Sabatini, A., Conte, L., (2007). Volatile Profile of Croatian Lime Tree (*Tilia* sp.), Fir Honeydew (*Abies alba*) and Sage (*Salvia officinalis*) Honey. Food Technology & Biotechnology 45(2), 156-165.

Masterova, I., Uhrin, D., Kettmann, V., Suchy, V., (1989). Phyto chemical study of *Salvia officinalis*. Chemical Papers 43(6), 797—803.

Natic, M.M., Dabic, D.C., Papetti, A., Fotiric Aksic, M.M., Ognjanov, V., Ljubojevic, M., Tesic, Z., (2015). Analysis and characterisation of phytochemicals in mulberry (Morus alba L.) fruits grown in Vojvodina, North Serbia. Food Chem 171, 128-136.

Patiny, L., Borel, A., (2013). ChemCalc: A Building Block for Tomorrow's Chemical Infrastructure. Journal of Chemical Information and Modeling 53(5), 1223-1228.

Persano Oddo, L., Bogdanov, S., (2004). Determination of honey botanical origin: problems and issues. Apidologie 35, S2-S3.

Piana, M., Persano Oddo, L., Bentabol, A., Bruneau, E., Bogdanov, S., Guyot Declerck, C., (2004). Sensory analysis applied to honey: state of the art. Apidologie 35(Suppl. 1), S26-S37.

Piazza, M., Persano Oddo, L., (2004). Bibliographic review of main unifloral European honeys. Apidologie 35, S94-S111.

Piljac-Žegarac, J., Stipčević, T., & Belščak, A., (2009). Antioxidant properties and phenolic content of different floral origin honeys. Journal of ApiProduct and ApiMedical Science 1(2), 43-50.

Primorac, L., Flanjak, I., Kenjerić, D., Bubalo, D., Topolnjak, Z., (2011). Specific Rotation and Carbohydrate Profile of Croatian Unifloral Honeys. Czech Journal of Food Sciences 29(5), 515-519.

Ricciardelli D'Albore, G., Galarini, R., (2000). Mediterranean Melissopalynology. Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia Italy.

Šugar, I., Gaži-Baskova, V., Trinajstić, I., Horvatić-Hodak, N., Lovrić, A., Horvatić, S., Kutleša, L., (1983). Vegetacijska karta SR Hrvatske, in: Botanički zavod Prirodoslovno-matematički fakultet (Ed.). Vojnogeografski institut Beograd, Zagreb.

Terrab, A., Hernanz, D., Heredia, F.J., (2004). Inductively coupled plasma optical emission spectrometric determination of minerals in thyme honeys and their contribution to geographical discrimination. J Agric Food Chem 52(11), 3441-3445.

The Council of the European Union, (2002). Council Directive 2001/110/EC of 20 December 2001 relating to honey, in: Union, T.C.o.t.E. (Ed.). Official Journal of the European Communities, pp. 47-49.

Tomás-Barberán, F.A., Grayer-Barkmeijer, R.J., Gil, M.I., Harborne, J.B., (1988). Distribution of 6-hydroxy-, 6-methoxy- and 8-hydroxyflavone glycosides in the labiatae, the scrophulariaceae and related families. Phytochemistry 27(8), 2631-2645.

Tomás-Barberán, F.A., Martos, I., Ferreres, F., Radovic, B.S., Anklam, E., (2001). HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. Journal of the Science of Food and Agriculture 81(5), 485-496.

Truchado, P., Ferreres, F., Bortolotti, L., Sabatini, A.G., Tomas-Barberan, F.A., (2008). Nectar Flavonol rhamnosides are floral markers of acacia (Robinia pseudacacia) honey. Journal of Agricultural and Food Chemistry 56(19), 8815-8824.

Von der Ohe, W., Persano Oddo, L., Piana, M., Morlot, M., Martin, P., (2004). Harmonized methods of melissopalynology. Apidologie 35, S18-S25.

Wang, M., Li, J., Rangarajan, M., Shao, Y., LaVoie, E.J., Huang, T.-C., Ho, C.-T., (1998). Antioxidative Phenolic Compounds from Sage (Salvia officinalis). Journal of Agricultural and Food Chemistry 46(12), 4869-4873.

Zgórka, G., Głowniak, K., (2001). Variation of free phenolic acids in medicinal plants belonging to the Lamiaceae family. Journal of Pharmaceutical and Biomedical Analysis 26(1), 79-87.

Figure Captions

Fig. 1. (**A**) PC scores plot of the honey samples; (**B**) Loadings plot of the honey samples.

Fig. S1. Base peak chromatogram of Common sage honey (sample No. **SH2**) extract. Peak numbers corresponds to those in **Table 3**: (**2**) gallocatechin, (**3**) salvianic acid aA, (**4**) protocatechuic acid, (**7**) epigallocatechin, (**9**) catechin, (**11**) chlorogenic acid, (**12**) *p*-hydroxybenzoic acid, (**13**) feruloyl-hexoside, (**14**) epicatechin, (**17**) coumaroyl-hexoside, (**22**) gentistic acid, (**23**) luteolin-rutinoside, (**24**) isorhamnetin-rutinoside, (**25**) quercetin-hexoside, (**27**) *p*-coumaric acid, (**28**) taxifolin, (**30**) rosmarinic acid, (**35**) *trans*, *trans*-abscisic acid, (**37**) monohydroxybenzoic acid, (**42**) sakuranetin, (**44**) kaempferol, and (**45**) rhamnetin.

Table 1 Apiary locations of the sage honey sample's production. Melissopalynological and sensory assessment of unifloral sage (*Salvia officinalis* L.) honey samples deriving from the North Croatian Littoral.

Sample	Location	Year	Melissopalynological assessment of honey samples	Sensory assessment of honey samples	Compliance to sage honey unniflorality
SH1	**Croatia, Cres**	2013	D	Fair	Complies
SH2	**Croatia, Eastern Istria**	2013	B	Good	Complies
SH3	**Croatia, Cres**	2013	C	Fair	Complies
SH4	**Croatia, Rab**	2012	C	Fair	Complies
SH5	**Croatia, Cres**	2012	C	Good	Complies
SH6	**Croatia, Krk**	2011	B	Good	Complies
SH7	**Croatia, Klenovica**	2011	C	Good	Complies
SH8	**Croatia, Krk**	2011	B	Fair	Complies
SH9	**Croatia, Cres**	2010	C	Good	Complies
SH10	**Croatia, Krk**	2010	B	Good	Complies
SH11	**Croatia,Cres**	2010	D	Good	Complies
SH12	**Croatia, Krk**	2010	C	Fair	Complies
SH13	**Croatia, Krk**	2009	C	Good	Complies
SH14	**Croatia, Cres**	2009	C	Fair	Complies
SH15	**Croatia, Kraljevica**	2012	B	Good	Complies
SH16	**Croatia, Cres**	2010	C	Fair	Complies
SH17	**Croatia, Cres**	2012	C	Fair	Complies
SH18	**Croatia, Krk**	2012	B	Good	Complies

Pollen frequency classes:

A - "Predominant pollen" (more than 45 % of the pollen grains);

B - "Secondary pollen" (16–45 %);

C - "Important minor pollen" (3–15 %);

D - "Minor pollen" (less than 3 %).

Table 2 Presence of polyphenolics in the sage (*Salvia officinalis* L.) honeys; number of identified compound, target compounds, mean expected retention times, exact mass, calculated mass, mean mass accuracy (ppm), and MS/MS fragments.

Peak No	Compounds	t_R, min	Exact mass, $[M–H]^-$	Calculated mass $[M–H]^-$	Δ pm	MS/MS fragments
1	**Gallic acid**[a]	2.55	169.01392	169.01425	1.95	125
2	**Gallocatechin**[a]	3.96	305.06583	305.06668	2.79	219, 261
3	**Salvianic acid A**	3.97	197.04520	197.04555	1.78	179, 153, 123
4	**Protocatechuic acid**[a]	4.34	153.01903	153.01933	1.96	109
5	**Chlorogenic acid isomer 1**	4.48	353.08716	353.08781	1.84	191, 179, 146
6	**Caffeoyl-hexoside**	4.52	341.08716	341.08781	1.91	179
7	**Epigallocatechin**[a]	4.65	305.06589	305.06668	2.59	219, 261
8	**Dimethoxybenzoic acid**	4.75	181.05025	181.05063	2.10	151, 137
9	**Catechin**[a]	4.92	289.07095	289.07176	2.80	159, 123
10	**Eriodictyol-rutinoside**	5.01	595.16644	595.16684	0.67	449, 287
11	**Chlorogenic acid**[a]	5.04	353.08682	353.08781	2.80	191, 179, 146
12	***p*-Hydroxybenzoic acid**[a]	5.09	137.02425	137.02442	1.24	93
13	**Feruloyl-hexoside isomer 1**	5.30	355.10229	355.10346	3.29	193
14	**Epicatechin**[a]	5.32	289.07114	289.07176	2.14	159, 123
15	**Gallocatechin gallate**[a]	5.34	457.07703	457.07763	1.31	305
16	**Chlorogenic acid isomer 2**	5.37	353.08710	353.08781	2.01	191, 179, 146
17	**Coumaroyl-hexoside**	5.39	325.09213	325.09289	2.34	163
18	**Epigallocatechin gallate**[a]	5.46	457.0769	457.07763	1.60	305
19	**Caffeic acid**[a]	5.48	179.03476	179.03498	1.23	135, 161
20	**Feruloyl-hexoside isomer 2**	5.61	355.10260	355.10346	2.42	193
21	**Rutin**[a]	5.94	609.14490	609.14611	1.99	463, 301
22	**Gentistic acid**[a]	5.96	153.01900	153.01933	2.16	109
23	**Luteolin-rutinoside**	5.97	593.15045	593.15119	1.25	447, 285
24	**Isorhamnetin-rutinoside**	6.03	623.16040	623.16176	2.18	461, 315
25	**Quercetin-hexoside**	6.07	463.08691	463.08820	2.79	301
26	**Ellagic acid**[a]	6.16	300.99847	300.99899	1.73	283, 200, 175
27	***p*-Coumaric acid**[a]	6.25	163.03984	163.04007	1.41	119

28	**Taxifolin**	6.47	303.05023	303.05103	2.64	285, 269, 255,217
29	**Ferulic acid**[a]	6.70	193.05014	193.05063	2.54	175, 139
30	**Rosmarinic acid**[a]	6.79	359.07635	359.07724	2.48	197, 179, 161
31	**Apigenin-rutinoside isomer**	6.83	577.15521	577.15628	1.85	431, 269
32	**Apigenin-hexoside isomer 1**	6.92	431.09775	431.09837	1.44	269
33	**Luteolin-hexoside**	6.93	447.09274	447.09329	1.23	285
34	**Eriodictyol**	7.14	287.05551	287.05611	2.09	125
35	***trans, trans*-Abscisic acid**	7.44	263.12814	263.12888	2.81	191, 179
36	**Apigenin-hexoside isomer 2**	7.52	431.09778	431.09837	1.37	269
37	**Monohydroxybenzoic acid**	7.70	137.02423	137.02442	1.39	93
38	***cis, trans*-Abscisic acid**[a]	7.73	263.12833	263.12888	2.09	191, 179
39	**Luteolin**[a]	7.75	285.03989	285.04046	2.00	213, 151
40	**Quercetin**[a]	7.80	301.03445	301.03538	3.09	151, 179, 121
41	**Resveratrol**[a]	7.85	227.07056	227.07137	3.57	209
42	**Sakuranetin**	8.06	285.07623	285.07685	2.17	133
43	**Apigenin**[a]	8.44	269.04477	269.04555	2.90	149, 151, 173, 183
44	**Kaempferol**[a]	8.57	285.03970	285.04046	2.67	199, 161, 151, 135
45	**Rhamnetin**	8.57	315.04996	315.05103	3.40	300, 165, 121
46	**Hispidulin**	8.66	299.05527	299.05611	2.81	284
47	**Pinobanksin**[a]	8.67	271.06067	271.06120	1.96	253, 243, 165, 151, 107
48	**Isorhamnetin**	8.73	315.05057	315.05103	1.46	300, 151, 107
49	**Hesperetin**[a]	8.77	301.07101	301.07176	2.49	271, 161
50	**Quercetin dimethyl ether 1**	8.98	329.06656	329.06668	0.36	315, 165
51	**Quercetin dimethyl ether 2**	9.64	329.06586	329.06668	2.49	315, 166
52	**Pinostrobin**[a]	9.83	269.08121	269.08193	2.68	151, 179
53	**Prenyl caffeate**	9.85	247.09703	247.09758	2.23	135, 179
54	**Chrysin**[a]	10.07	253.05009	253.05063	2.13	101, 151, 181, 209, 143
55	**Pinocembrin**[a]	10.09	255.06563	255.06628	2.55	213, 211, 151
56	**Acacetin**	10.14	283.06094	283.06120	0.92	268, 133, 151, 107
57	**Caffeic acid phenethyl ester (CAPE)**	10.15	283.09714	283.09758	1.55	135, 179
58	**Galangin**[a]	10.25	269.04489	269.04555	2.45	151, 183
59	**Genkwanin**	10.62	283.06042	283.06120	2.76	268, 239, 211

60	**Carnosol**[a]	11.72	329.17487	329.17583	2.92	311, 296
61	**Carnosic acid**[a]	12.81	331.19061	331.19148	2.63	287, 269

[a] Confirmed using available standards.

Table 3 Quantification of individual polyphenolics (mg/kg), radical scavenging activity (*RSA*) and total phenolic content (*TPC*) in the sage (*Salvia officinalis* L.) honeys.

	SH1	**SH2**	**SH3**	**SH4**	**SH5**	**SH6**	**SH7**	**SH8**	**SH9**	**SH10**	**SH11**	**SH12**	**SH13**	**SH14**	**SH15**	**SH16**	**SH17**	**SH18**
GaA	-	0.15	0.13	0.12	0.12	0.18	0.16	0.19	-	0.13	-	0.12	-	0.17	0.13	0.15	0.12	-
GC	-	0.49	0.15	0.19	0.16	0.16	-	-	0.16	0.22	0.16	0.15	-	-	-	0.20	0.26	0.37
PrA	0.34	0.70	0.74	0.54	0.25	0.34	0.31	0.68	0.62	0.48	0.49	0.14	0.31	0.42	0.33	0.39	0.44	0.68
EGC	0.12	-	0.46	0.10	0.08	0.12	0.08	0.10	0.14	0.14	0.09	0.07	0.16	0.08	0.08	-	0.24	0.16
GeA	-	-	-	-	-	-	-	0.13	-	-	-	-	-	-	0.01	-	-	0.02
HBA	1.89	1.93	1.61	1.25	1.82	1.45	3.28	2.18	1.36	1.82	1.20	0.81	1.34	1.55	2.06	1.73	1.66	2.36
ChA	-	0.04	0.01	0.10	0.22	-	0.02	0.05	0.26	0.09	0.04	0.03	0.07	-	-	0.10	0.03	-
C	0.12	0.03	0.15	0.09	-	-	-	-	0.05	0.01	0.04	-	0.05	-	-	0.01	0.06	-
CaA	0.48	1.89	0.56	0.37	0.48	0.59	0.57	0.92	0.54	0.64	0.61	0.62	0.37	0.38	0.47	0.70	0.55	0.26
GCG	0.82	0.74	0.75	-	-	-	1.15	1.05	0.72	0.70	0.72	0.69	0.81	0.69	0.71	0.69	0.73	1.03
EC	0.10	-	0.03	0.06	-	-	-	-	-	-	-	-	-	-	-	0.05	0.07	-
CoA	2.73	3.11	3.45	1.89	2.01	1.36	3.62	2.78	2.81	1.39	1.82	2.51	1.03	0.77	0.92	1.62	2.68	1.10
FeA	0.64	1.54	3.09	0.97	0.44	0.50	1.01	4.39	1.31	0.34	0.51	0.77	0.60	0.16	0.83	0.53	0.67	1.40
RoA	-	0.21	0.01	0.21	0.37	0.30	0.33	0.05	0.18	0.09	0.15	0.23	0.14	0.27	0.25	0.14	0.26	0.29
EG	0.94	1.2	0.97	0.8	0.7	1.11	1.0	0.9	0.8	-	0.9	1.2	0.9	0.5	1.0	1.2	1.1	1.6

CG		5		5	9		0	6	0		6	6	5	8	7	8	4	1
AbA	0.35	1.06	1.89	0.54	0.74	1.09	0.73	3.99	0.61	0.26	0.48	0.86	0.39	0.64	1.64	0.69	0.46	1.59
RES	-	0.11	-	-	0.22	-	-	-	-	-	-	-	-	-	-	0.08	0.46	-
KAE	0.14	0.18	0.24	0.78	0.46	0.16	0.20	0.21	0.51	0.38	0.65	0.03	0.38	0.18	0.26	0.54	0.55	0.28
PNB	-	2.35	0.35	0.33	0.63	1.57	1.00	1.82	0.82	1.30	1.00	2.26	0.44	0.58	1.10	1.14	0.24	0.21
QUE	0.07	0.38	0.14	0.58	0.23	0.12	0.17	0.33	0.36	1.05	0.36	0.11	0.32	0.36	0.14	0.59	0.45	0.30
CHR	0.06	1.98	0.41	0.27	0.23	0.87	0.48	0.95	0.47	0.81	0.73	1.50	0.42	0.47	0.90	0.92	0.25	0.09
PNS	-	0.34	-	-	-	0.07	0.04	0.19	0.01	0.07	0.03	0.19	-	-	0.05	0.04	-	-
PNC	-	0.80	0.09	0.05	0.15	0.51	0.22	0.46	0.28	0.45	0.35	0.72	0.15	0.17	0.46	0.43	-	-
HES	-	0.84	0.19	0.06	0.20	0.37	0.11	0.38	0.27	0.33	0.20	0.52	0.04	0.09	0.36	0.36	-	-
GLN	-	0.39	0.01	-	0.01	0.11	0.07	0.23	0.04	0.12	0.07	0.22	0.01	-	0.09	0.09	-	-
TPC	553.98	485.86	424.92	591.08	522.33	484.91	471.03	509.11	538.51	560.72	747.49	417.73	525.39	444.97	379.54	545.40	585.28	208.51
RSA	819.75	627.44	526.81	770.65	548.55	571.41	541.69	627.79	610.38	586.04	894.87	474.17	641.38	675.23	416.81	626.20	770.11	351.20

GaA – Gallic acid; **GC** – Gallocatechin; **PrA** – Protocatechuic acid; **EGC** – Epigallocatechin; **GeA** – Gentisic acid; **HBA** – *p*-Hydroxybenzoic acid; **ChA** – Chlorogenic acid; **C** – Catechin; **CaA** – Caffeic acid; **GCG** - Gallocatechin gallate; **EC** – Epicatechin; **CoA** – *p*-Coumaric acid; **FeA** – Ferulic acid; **RoA** – Rosmarinic acid; **EGCG** - Epigallocatechin gallate; **AbA** – *cis, trans*-Abscisic acid; **RES** – Resveratrol; **KAE** – Kaempferol; **PNB** – Pinobanksin; **QUE** – Quercetin; **CHR** – Chrysin; **PNS** – Pinostrobin; **PNC** – Pinocembrin; **HES** – Hesperetin; **GLN** – Galangin; **TPC** – Total phenolic content (mg GEA/kg); **RSA** – Radical scavenging activity (μmol TE/kg).

Table 4 Quantification of the sugars and sugar alcohols in the sage (*Salvia officinalis* L.) honeys (g/kg).

	SH1	SH2	SH3	SH4	SH5	SH6	SH7	SH8	SH9	SH10	SH11	SH12	SH13	SH14	SH15	SH16	SH17	SH18
ERY	0.04	0.07	0.05	0.05	0.02	0.07	0.06	0.07	0.05	0.07	0.09	0.69	0.06	0.07	0.18	0.12	0.11	0.09
SOR	0.04	0.13	0.03	0.06	0.09	0.08	0.30	0.33	0.20	0.06	0.06	0.03	0.04	0.02	0.18	0.05	0.05	0.02
TRE	1.23	5.70	2.71	2.07	5.34	0.21	5.07	2.76	1.45	5.08	1.82	0.75	1.31	2.39	1.41	0.14	0.63	1.02
ARA	0.04	0.15	0.05	0.10	0.10	0.37	0.12	0.07	0.06	0.07	0.10	0.05	0.09	0.05	0.05	0.06	0.08	0.03
GLU	305.03	272.07	280.3	253.24	212.95	206.81	108.89	245.39	200.35	263.82	227.38	240.15	244.46	252.72	271.21	262.22	277.59	263.08
FRU	399.41	464.01	440.3	420.83	393.63	475.99	480.76	437.51	442.23	462.32	464.06	489.06	461.97	455.68	461.76	464.87	447.35	471.03
SUC	30.41	28.78	11.02	15.69	14.17	14.27	20.71	14.03	11.87	16.54	21.4	27.08	25.64	19.01	11.21	18.59	16.34	16.16
TUR	1.34	0.12	0.32	0.68	0.6	0.83	1.13	0.76	0.88	0.65	0.87	0.82	0.82	0.90	0.11	0.77	1.16	1.06
GLY	1.51	0.11	0.16	0.09	0.12	0.17	0.12	0.16	0.03	0.19	0.10	0.05	0.08	0.07	0.17	0.12	0.12	0.13
GAL	0.06	0.06	0.04	0.09	0.46	0.33	0.20	0.36	0.02	0.07	0.08	0.03	0.02	0.03	0.2	0.06	0.07	0.03
iMAL	4.45	3.11	5.31	14.6	3.63	7.13	7.68	5.40	3.95	12.61	12.34	3.52	7.44	8.51	8.28	10.22	11.62	9.98
iMALt	1.44	2.88	0.73	4.66	2.55	2.76	1.24	1.48	1.11	3.79	5.66	1.11	0.33	0.36	0.31	2.41	3.91	2.44
MAL	6.05	3.74	5.92	16.85	8.76	9.32	11.91	7.32	7.38	11.77	11.09	7.40	8.98	10.06	8.33	11.49	12.31	9.91
MALt	0.11	0.06	0.01	0.02	0.02	0.05	0.06	0.08	0.06	0.04	0.07	0.08	0.09	0.07	0.02	0.09	0.08	0.08
SUM	751.16	780.99	746.95	729.03	642.44	718.39	638.25	715.72	669.64	777.08	745.12	770.82	751.33	749.94	763.42	771.21	771.42	775.06
FRU/GLU	1.31	1.71	1.57	1.66	1.85	2.30	4.42	1.78	2.21	1.75	2.04	2.04	1.89	1.80	1.70	1.77	1.61	1.79

MAL/iMAL	1.36	1.20	1.11	1.15	2.41	1.31	1.55	1.35	1.87	0.93	0.90	2.10	1.21	1.18	1.01	1.13	1.06	0.99

ERY – Erythritol; **SOR** – Sorbitol; **TRE** – Trehalose; **ARA** – Arabinose; **GLU** – Glucose; **FRU** – Fructose; **SUC** – Sucrose; **TUR** – Turanose; **GLY** – Glycerol; **GAL** – Galactitol; **iMAL** – Isomaltose; **iMALt** – Isomaltotriose; **MAL** – Maltose; **MALt** – Maltotriose; **SUM** – Summary of quantified sugars and sugar alcohols; ***FRU/GLU*** – Fructose/Glucose ratio; ***MAL/iMAL*** – Maltose/Isomaltose ratio.

Table 5 Quantification of the minerals in the sage (*Salvia officinalis* L.) honeys (mg/kg).

	SH 1	SH 2	SH 3	SH 4	SH 5	SH 6	SH 7	SH 8	SH 9	SH 10	SH 11	SH 12	SH 13	SH 14	SH 15	SH 16	SH 17	SH 18
Al	0.095	0.433	0.431	0.419	0.145	0.023	<*LOQ*	0.261	0.505	0.266	0.387	<*LOQ*	0.306	0.883	0.059	0.263	0.149	0.111
As	0.003	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	0.006	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	0.013	<*LOQ*	<*LOQ*
B	1.270	2.376	1.134	1.290	1.876	2.760	2.113	2.599	1.907	1.388	2.086	2.201	2.174	1.774	3.029	2.140	1.455	1.681
Ba	0.051	0.094	0.084	0.068	0.076	0.066	0.059	0.076	0.067	0.064	0.072	0.076	0.064	0.071	0.071	0.060	0.069	0.062
Ca	23.823	49.894	50.427	50.471	36.787	23.591	31.557	47.122	34.375	32.747	39.980	20.409	35.098	22.978	36.471	30.205	30.493	20.885
Cd	0.003	0.004	0.003	0.004	0.003	0.003	0.003	0.004	0.003	0.003	0.003	0.003	0.003	0.004	0.004	0.003	0.003	0.003
Co	<*LOQ*	<*LOQ*	<*LOQ*	0.012	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*
Cr	0.004	0.005	0.004	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	0.015	0.006	0.010	0.007	<*LOQ*	0.013	0.009	<*LOQ*	<*LOQ*	<*LOQ*	0.003
Cu	0.101	0.299	0.187	0.126	0.220	0.198	0.283	0.173	0.233	0.155	0.219	0.221	0.166	0.187	0.313	0.152	0.124	0.109
Fe	0.247	0.928	0.527	0.533	0.457	0.442	0.274	0.853	0.371	0.479	2.278	0.400	2.494	0.806	0.195	0.382	0.248	0.496
K[a]	2.151	1.281	1.838	2.037	1.313	0.844	0.909	0.982	1.026	1.116	1.873	0.592	1.300	0.730	1.213	1.145	2.107	0.675
Li	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*	<*LOQ*
M	5.0	26.	11.	10.	7.9	7.9	8.9	25.	8.0	11.	8.7	5.9	7.1	9.9	10.	7.1	5.7	10.

g	23	412	762	123	30	32	03	895	40	190	92	37	07	59	519	46	26	168
Mn	0.196	0.775	1.064	0.598	0.277	0.137	0.237	1.063	0.370	0.296	0.645	0.169	0.315	0.267	0.277	0.326	0.270	0.185
Mo	*<LOQ*	0.003	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*
Na	8.379	6.966	0.115	38.935	28.630	7.807	3.201	12.341	6.638	6.519	8.400	18.695	6.020	5.614	10.434	5.139	17.005	1.174
Ni	0.019	0.033	0.028	0.024	0.008	0.018	0.015	0.053	0.019	0.026	0.033	0.024	0.032	0.071	0.034	0.008	0.008	0.149
P	34.568	101.564	37.327	44.222	41.339	36.455	40.287	56.050	32.730	49.512	39.074	33.433	33.637	41.744	48.823	32.050	35.386	39.013
Pb	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	0.073	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	0.026	*<LOQ*	*<LOQ*
S	26.132	43.389	27.427	29.321	26.227	23.605	27.715	33.115	20.125	91.506	28.139	19.258	22.438	23.161	34.543	21.435	24.583	16.327
Sb	*<LOQ*	0.006	*<LOQ*	*<LOQ*	0.029	0.009	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	0.017	*<LOQ*	0.048	0.004	*<LOQ*	*<LOQ*
Se	0.025	0.034	0.038	0.062	0.060	0.030	0.055	0.074	0.012	0.055	0.042	0.047	0.017	*<LOQ*	0.049	0.037	0.054	0.041
Sr	0.017	0.047	0.040	0.040	0.029	0.027	0.014	0.031	0.029	0.036	0.028	0.030	0.023	0.039	0.028	0.023	0.022	0.023
V	*<LOQ*	0.013	0.004	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	0.012	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*
Zn	1.119	0.687	1.161	1.417	1.029	0.203	0.573	0.594	0.873	1.081	7.190	0.272	2.215	0.241	0.488	1.228	0.785	5.887

LOQ = 0.0025 mg/kg;

[a] Amount of K is expressed as g/kg.