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*original scientific paper*

# Volatile Profile of Croatian Lime Tree (*Tilia* sp.), Fir Honeydew (*Abies alba*) and Sage (*Salvia officinalis*) Honey

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**Summary**

Volatile profiles of lime tree (*Tilia* sp.), fir honeydew (*Abies alba*) and sage (*Salvia officinalis*) honey produced in Croatia have been studied by using headspace solid phase microextraction (HS-SPME) followed by gas chromatography-mass spectrometry (GC-MS) analysis. Melissopalynological and sensory characterization have been performed in order to check the reliability of botanical origin of the samples. In case of sage honey, sensory characteristics are reported for the first time and are rather uniform including: colour characterized as beige to jade, depending on the consistency; odour characterized as between light and medium intensity, slightly pungent and wooden; taste characterized as low sweetness, expressive acidity and apple caramel, with persistent fruity aftertaste. Characteristic volatile profiles of the analyzed honeys are described. Taking into consideration similarities with lime and fir honey volatile profiles reported in literature, characteristic volatile compounds resulting from qualitative data evaluation are proposed. Sage honey volatile profile has been reported for the first time and it was found quite different compared to the other studied honeys showing the lowest number of peaks among the studied honeys, 34. Several compounds belonging to the sage honey volatile profile have been identified for the first time in honeys. They include tetrahydro-2,2,5,5-tetramethylfuran, 3-hexenyl ester of butanoic acid, 2-methylbenzene, maltol, methyl ester of 3-furanocarboxylic acid and benzeneacetic acid. Based on the obtained results and with the lack of comparative literature data, they are proposed as characteristic volatiles for the volatile pattern of sage honey.

*Key words:* sage honey, lime tree honey, fir honeydew honey, volatiles, solid phase microextraction (SPME), sensory characterization

## Introduction

Honey is one of the oldest foods used by humans. The appreciation of honey quality varied during history based mostly on sensory characteristics and botanical and geographical origin. The assessment of honey's botanical and geographical origin is very complex (*1*). It includes the results of microscopic, chemical and sensory analyses. This kind of evaluation is especially prob-

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lematic when different botanical species (more or less nectar and pollen productive) are involved and when clear and precise pollen analysis is very difficult (*2,3*). Due to high complexity of these methods and the interpretation of results (*4*), the attention is turned toward alternative methods of control and new approaches are proposed for the characterization of the unifloral honeys (*5*), often dealing with identification of chemical »markers« both in the non-volatile fraction (*e.g.* aminoacids, organic acids, *etc.*) (*6–9*), as well as in the volatile fraction of honeys. Unifloral honeys are mainly characterized by distinctive aroma deriving from botanical origin of nectar. That has encouraged the research of chemical composition of honey aroma fraction and typical compounds as markers of its origin. Continuous liquid/liquid extraction of volatiles and gas chromatography-mass spectrometry (GC-MS) has been used for unifloral honey characterization of honeys of certain geographic regions (*10–14*). Volatile compounds of different honeys were studied by means of optimized Likens-Nickerson method (*15*). Studies of unifloral honey aroma fraction have also been conducted by means of dynamic headspace gas chromatography followed by MS detection (*16*). Several unifloral honeys were analyzed by headspace solid phase microextraction (HS-SPME) coupled with GC-MS system in order to establish simpler method that can be applied for the characterization of different honeys based on the presence of specific compounds (*17–19*). The aim of this work was to study volatile profiles of fir honeydew (*Abies alba*) honey, sage (*Salvia officinalis*) honey and lime tree (*Tilia* sp.) honey from Croatia using HS-SPME followed by GC-MS analysis.

While fir honeydew and lime tree honey have certain literature background about their volatile composition (*17,18,20*), sage honey has been poorly studied. This floral honey is produced in a relatively small quantity because of limited climatic and pedological conditions where sage grows as predominant botanical species (narrow littoral stripe of the Mediterranean). Nevertheless, sage honey represents an interesting object of research because of the growing market demand related to specific taste and aroma, and to consumer's perception about its medicinal properties (*21*). Similar interest is present for the fir honey, the production of which is also limited because of its dependency both on the activity of the plant sucking insects and on the limited fir area.

## Materials and Methods

### *Sampling*

Seventeen honey samples of three botanical origins (fir honeydew, sage and lime tree honey) from 13 locations in Croatia have been studied in this research (Table 1).

Locations of honey production were chosen according to the data obtained from detailed vegetation maps of Croatia (*22*). Sampling was conducted directly from the hive sites or in producer's filling processing plants using standard sampling method described in Official Gazette of The Republic of Croatia (*23*). Hive sites were situated in the middle of the respective vegetation areas (sage dominating area, lime tree dominating area, fir dominating area), in order to obtain honeys of the known botanical origin. Samples were put in glass containers until full, sealed and stored in refrigerator at 4 °C until analyzed.

Table 1. List of honey samples, year and location of production

| Sample code | Honey samples | Year of production | Location of production |
|---|---|---|---|
| LT1 | Lime tree 1 | 2000 | Kikovica, Ostrovica |
| LT2 | Lime tree 2 | 2000 | Kikovica, Ostrovica |
| LT3 | Lime tree 3 | 2000 | Studena |
| LT4 | Lime tree 4 | 2000 | Fuzine |
| LT5 | Lime tree 5 | 2000 | Krasica |
| FH1 | Honeydew 1 | 1999 | Fuzine |
| FH2 | Honeydew 2 | 2000 | Moravice |
| FH3 | Honeydew 3 | 2000 | Moravice-2 |
| S1 | Sage 1 | 2000 | Osor-1, Cres |
| S2 | Sage 2 | 1999 | Stivan-1, Cres |
| S3 | Sage 3 | 2001 | Stivan-2, Cres |
| S4 | Sage 4 | 2001 | Stivan-1, Cres |
| S5 | Sage 5 | 2001 | Osor-2, Cres |
| S6 | Sage 6 | 2001 | Osor-3, Cres |
| S7 | Sage 7 | 2001 | Krizisce, Cres |
| S8 | Sage 8 | 2001 | Draga, Rijeka |
| S9 | Sage 9 | 2001 | Stivan-1, Cres |

LT, FH and S: lime tree, fir honeydew and sage honey, respectively

### *Melissopalynological analyses and sensory evaluation*

Melissopalynological analyses and sensory evaluations were conducted at the National Institute of Apiculture, Bologna, Italy. Melissopalynological analyses were performed according to the method described by Loveaux *et al.* (*24*), while sensory evaluation of botanical origin matching was done according to the International Organization for Standardization (ISO) methods (*25,26*) by a panel of assessors specialized for sensory analysis of honey.

### *Sample preparation for HS-SPME analyses*

Sampling preparation was performed according to the method reported previously by Piasenzotto *et al.* (*18*), slightly modified. A sample of honey ((3±0.001) g) was weighed in 50-mL vial and 0.5 g of anhydrous sodium sulphate ($Na_2SO_4$, BDH Laboratory supplies, Poole, UK) was added. Its amount was calculated using stechiometric calculation (with adding a little excess in salt mass) on the basis of determined honey water content according to refractometric method described in »Harmonised Methods of the International Honey Commission« (*4*). The vial was sealed with a Teflon coated silicon septum (Alltech, Milano, Italy). Vial, septum and anhydrous sodium sulphate had previously been conditioned at 80 °C overnight in order to remove all foreign volatile compounds. Sealed vial was conditioned in water bath at 40 °C for 20 min and rotated twice during that period. Solid phase micro extraction (SPME) was carried out by means of a fibre coated with cross-linked divinylben-

zene/carboxen/polydimethylsiloxane (DVB/Car/PDMS), 50/30 μm thick and 2 cm long (Supelco, Bellefonte, PA, USA), conditioned prior to use by heating in the injection port of a GC system under the conditions recommended by the manufacturer. Fibre was exposed to the sample's headspace at 40 °C for 20 min, then it was used for GC-MS injection. Cleaning of the fibre was applied after every injection by heating it for 15 min in the injection port of the GC system at the same temperature as used for analysis.

### *GC-MS analyses*

Thermal desorption of volatiles trapped on the fibre was carried out at 250 °C in the GC injector port of a Varian model 3400 (Varian, Torino, Italy) coupled with Varian Saturn ion trap detector. An HP-INNOWax capillary column 30 m long, with inner diameter of 0.32 mm and coated with 0.5 μm thick stationary phase (Hewlett Packard) was used. Column temperature was held at 50 °C for 4 min, then increased to 230 °C at 10 °C/min, then held at 230 °C for 10 min and finally increased to 250 °C at 10 °C/min. Splitless system was used for injections: the split valve was opened 3 min after the injection (50:1 split ratio). Transfer line temperature was 250 °C, ion trap temperature was 170 °C, the carrier gas (He) flow rate was 1.5 mL/min. Electron impact spectra (70 eV) were recorded at 1 scan/s with the filament emission of 10 μA. A delay in acquisition of MS was set to 5 min in order to avoid any possible damage to the system (source). A standard mixture of hydrocarbons, from *n*-$C_8$ to *n*-$C_{32}$ (Sigma-Aldrich, Milano, Italy) was used for relative retention index (RI) calculation. Tentative identification of the volatile compounds was achieved by comparing mass spectra of the unknown peaks with those stored in the NIST90, WILEY5 and FFCII libraries and with those recorded for authentic standards, when available (*18*). Retention indices were calculated for the HP-INNOWax capillary column in accordance with modified Van den Dool and Kratz equation (*27*). Repeatability of the method was calculated from the peak areas obtained from eight consecutive analyses of the same honey sample.

## Results and Discussion

Melissopalynological analyses and sensory evaluation were performed in order to check the reliability of botanical origin of samples. Relatively low level of available scientific data about sage (*S. officinalis*) honey composition and its quality were the reason for including higher number of sage honey samples compared to other studied honeys. Microscopic parameters had confirmed the botanical origin of sampled honeydew (*A. alba*), and lime tree (*Tilia* sp.) honeys. Melissopalynological analyses of all sage honey samples showed low frequency of presence of sage (*S. officinalis*) pollen grains compared to pollen frequencies of other monofloral honeys. In most of the cases (5 of 9 sage honey samples), frequencies of presence of sage pollen grains were attributed to the class of »isolated pollen types«, meaning less than 3 % of sage pollen grains in the insoluble sediment. Overall medium percentage of sage flower pollen was around 10 %, but pollen percentage values varied significantly from sample to sample.

Sensory evaluation of botanical origin matching compared to already available standard honey sensory profile (*28*) was positive in case of sampled honeydew (*A. alba*), and lime tree (*Tilia* sp.) honeys.

Honeydew honey samples had characteristic slightly salted taste and caramel smell, while lime honey samples were all characterized by typical balsamic and medicinal smell. Same evaluation could not be performed in case of sage honey samples since standard sensory profile has not yet been established. Low frequency of sage (*S. officinalis*) pollen grains compared to pollen frequencies of other monofloral honeys could be attributed to the natural hypopollenic production of the sage – Mediterranean plant with low pollen quantity in flowers. Dominant pollen grains were identified as belonging to *Rhamnaceae*, *Castanea* and *Umbelliferae* botanical species of nectar producing plants, while *Quercus*, *Vitis*, *Fraxinus* and *Graminaceae* species were identified as pollen sources of non-nectar producing plants. Nevertheless, particulars of the vegetation map of Croatia (*22*) confirmed that sage honey samples were taken from the beehives deeply inside the sage-dominating vegetation zone. Furthermore, sampling took place in the middle of the sage flowering period (early May) when sage flowers were the main bee source of nectar. These two facts contributed to the authenticity of sage honey botanical origin.

### *Sensory analyses*

A series of characterization cards was drawn up on the basis of a wide sample collection of definite geographical and botanical origin (*28*). This has facilitated determination of conformity to the standard sensory profile for lime tree and fir honeydew honey samples, while it could not be performed for sage honey samples. In effect, since sage honey is a very particular type of honey, it requires a particular production and is not easily found, its sensory profile has not yet been defined. To be able to do that, a more conspicuous sample collection is requested. Results shown in Tables 2 and 3 proved that Croatian lime tree and fir honeys had standard sensory profiles established for these two types of honey (*1,29*).

Sensory properties of the studied sage honey samples were rather uniform, and they could be described as follows: colour – beige to jade (depending on the consistency); odour – between light and medium intensity, slightly pungent, wooden; taste – low sweetness, expressive acidity and apple caramel, with persistent fruity aftertaste (Table 4). These are the most frequent sensory properties of the sage honey samples which could be proposed as the standard sensory sage honey profile.

### *Composition of volatile fraction*

SPME was chosen as analytical approach because of its well known advantages, *e.g.* to be a quite solventless technique with reduced temperature application. It has widespread application to a number of different matrices. Application of SPME for honey characterization has been published by several authors (*17,18,30*). Yield of extraction and concentration of volatile compounds in the headspace can be improved by the addition of salt that

Table 2. Sensory evaluation of lime tree honey (LT) samples

| Sample code | Sensory evaluation | | | | |
|---|---|---|---|---|---|
| | Visual evaluation | | Olfactory evaluation | Flavour evaluation | Tactile characteristics evaluation |
| | Physical state | Colour | Odour | Taste | Consistency |
| LT1 | Homogenous crystallization | Ivory | Intensive, pungent, balsamic, mentholic | Normal sweetness, intensive aroma, fresh, mentholic, persistent | Soft, with bigger and smaller crystals that hardly melt |
| LT2 | Homogenous crystallization | Ivory | Intensive, pungent, balsamic, mentholic | Normal sweetness, intensive taste, fresh mentholic, persistent | Soft, with adhesive and melted crystals |
| LT3 | Homogenous crystallization | Light beige | Intensive, pungent, balsamic, mentholic | Normal sweetness, intensive aroma, fresh, mentholic, persistent | Compact, with fine, adhesive crystals |
| LT4 | Homogenous crystallization | Light beige | Intensive, pungent, balsamic, liquirice, mentholic | Normal sweetness, intensive aroma, fresh, mentholic, persistent | Soft, medium size and rough crystals |
| LT5 | Beginning of crystallization | Light beige | Intensive, pungent, balsamic, slightly medicinal (similar to drugstore) | Low sweetness, intensive aroma, strongly mentholic | Normal density |

Table 3. Sensory evaluation of fir honeydew honey (FH) samples

| Sample code | Sensory evaluation | | | | |
|---|---|---|---|---|---|
| | Visual evaluation | | Olfactory evaluation | Flavour evaluation | Tactile characteristics evaluation |
| | Physical state | Colour | Odour | Taste | Consistency |
| FH1 | Liquid, slightly opalescent | Dark jade | Between medium and highly intensive, balsamic, resemblance to resin | Low sweetness, medium intensity aroma, piquant | Normal density |
| FH2 | Liquid, opalescent | Dark jade | Between medium and highly intensive, balsamic, herbal, resemblance to resin | Low sweetness, medium intensity aroma, resembles olfactory characteristics | Optimal liquidity |
| FH3 | Homogenous crystallization | Brown | Intensive, balsamic, resemblance to resin | Low sweetness, medium intensity aroma, malt, as marmalade | Pasty, with rough, large size crystals |

Table 4. Sensory evaluation of sage honeys (S)

| Sample code | Sensory evaluation | | | | |
|---|---|---|---|---|---|
| | Visual evaluation | | Olfactory evaluation | Flavour evaluation | Tactile characteristics evaluation |
| | Physical state | Colour | Odour | Taste | Consistency |
| S1 | Homogenous crystallization | Light beige | Medium intensity, delicate, lightly spicy | Sweetness in normal margins, fresh, persistent retronasal, fruity, flowery taste | Soft, barely visible crystals |
| S2 | Homogenous crystallization | Light brown | Between medium and highly intensive, caramel, can be related to heather honey taste | Sweetness not too high, caramel, slightly resemblance to starch, cooked fruit | Soft, few large size and rough crystals |
| S3 | Homogenous crystallization | Beige | Between light and medium intensity, slightly pungent, wooden | Low sweetness, expressive acidity, apple caramel, retronasal fruity taste | Soft, no visible crystals |
| S4 | Beginning of crystallization | Jade | Between light and medium intensity, slightly pungent, wooden | Low sweetness, expressive acidity, apple caramel, fruity aftertaste | Optimal consistency |

Table 4. – continued

| Sample code | Sensory evaluation | | | | |
|---|---|---|---|---|---|
| | Visual evaluation | | Olfactory evaluation | Flavour evaluation | Tactile characteristics evaluation |
| | Physical state | Colour | Odour | Taste | Consistency |
| S5 | Beginning of crystallization | Jade | Between light and medium intensity, slightly pungent, wooden | Low sweetness, expressive acidity, apple caramel, fruity aftertaste | Optimal consistency |
| S6 | Liquid, opalescent | Jade | Between light and medium intensity, slightly pungent, wooden | Low sweetness, expressive acidity, apple caramel, fruity aftertaste | Optimal consistency |
| S7 | Liquid, opalescent | Light jade | Week, caramel, malt | Low sweetness, apple caramel, fruity aftertaste | Optimal consistency |
| S8 | Liquid, opalescent | Dark jade | Medium intensity, malt, similar to cooked fruit, wooden, slight resemblance to uric acid | Normal sweetness, expressive acidity, apple caramel, fruity aftertaste, very persistent that resembles Muscat grape variety | Optimal consistency |
| S9 | Liquid, opalescent | Light jade | Between light and medium intensity, slightly pungent, wooden | Low sweetness, normal acidity, apple caramel, sweet cotton | Optimal consistency |

could be performed on undiluted samples, or sample dilution could be followed by salt addition. Sodium sulphate and sodium chloride are the most frequently used salts. In this research, the first approach is carried out by simply adding sodium sulphate to undiluted honey sample in order to realize salting-out effect. On the other hand, possible differences in water content of different types of honey and different concentration and chemical nature of solutes cause different water activity ($a_w$) values. Sodium sulphate was also used in order to avoid any important influence of water. In several studies the application of different SPME fibres for honey volatile compound determination was described (*17–19*). Among different kinds of commercially available SPME fibres, DVB/Car/PDMS fibre was chosen because of the following considerations. Firstly, this »three-phase« fibre allows sampling of large spectra of honey volatile compounds characterized by widespread polarity and molecular mass. Secondly, higher yields were obtained when three-phase fibre was used because of its higher phase thickness and its longer layer (2 cm). This was important mainly in order to increase the amount of analytes that reach the MS system, resulting in better MS spectra. At the present state of the art of this research, just qualitative data are reported because of the lack of reference standards for all the 100 and more separated substances. Particular attention was given to blank trials as a complex series of peaks was obtained by HS-SPME followed by GC-MS analyses of lime tree honey (Fig. 1), fir honeydew honey and sage honey.

In order to discriminate irrelevant peaks depending on compounds from laboratory atmosphere or fibre fragmentation, laboratory atmosphere was tested with HS-SPME followed by GC-MS analysis for several times during research period. Based on these data it was possible to take into consideration only those peaks that represent intrinsic volatile compounds of the studied honey samples. In the context of the qualitative determination of volatile compounds and taking into consideration that HS-SPME followed by GC-MS is equilibrium-based method, it was important to verify its repeatability. Peaks deriving only from the compounds of the sample matrix, representing at least 1 % of the highest peak area, were taken into consideration. Relative standard deviation for areas of selected peaks ranged from 4.19 to 13.65 % and it proved that repeatability of the method was satisfactory. According to the qualitative data analyses, high similarity in the presence or absence of each volatile compound in the samples of the same botanical origin (minimum 2/3 of the samples) was noticed and shown in Table 5 (*14–19,31,32*). Some of these compounds may be considered as characteristic of botanical origin since they were not detected in the other two types of studied honeys. Peaks representing these compounds are marked with diagonal arrows as shown in Fig. 1 in the case of lime tree honey. The highest number of individual volatile compounds was obtained for lime tree honey (74), which is in accordance with the observation made by Radovic *et al.* (*16*). Fir honeydew and sage honey samples showed less complex volatile pattern (45 and 34 volatile compounds, respectively). Lime tree honey was found as characterized by several identified substances. Volatile compounds found only in lime tree honey samples, and already proposed by other authors as markers for lime tree honey were: *trans*-2-caren-4-ol (*16,18*), terpinene (*16,18*), rose oxide (*10,19*), 4-methyl-1-(1-methylethyl)--3-cylohexen-1-ol (4-terpinenol) (*18*), 1-(4-methylphenyl)-ethanone (*p*-methylacetophenone) (*16,18*), α-terpinen-7--al (*18*), 2-methyl-5-(methylethyl)phenol (carvacrol) (*33*), 5-methyl-2-(1-methylethyl)phenol (thymol) (*33*) and 1-methyl-4-(1-methylethyl)benzene (*p*-cymene) (*16*). Studies of the set of honey samples that did not include honeydew and sage honey samples suggested also borneol (*18*), benzenedicarboxylic acid derivative (*18*), and dimethyl styrene (*16,18*), as characteristic compounds for lime tree honey. Since in this study these substances were detected in fir honeydew honey samples, they should not

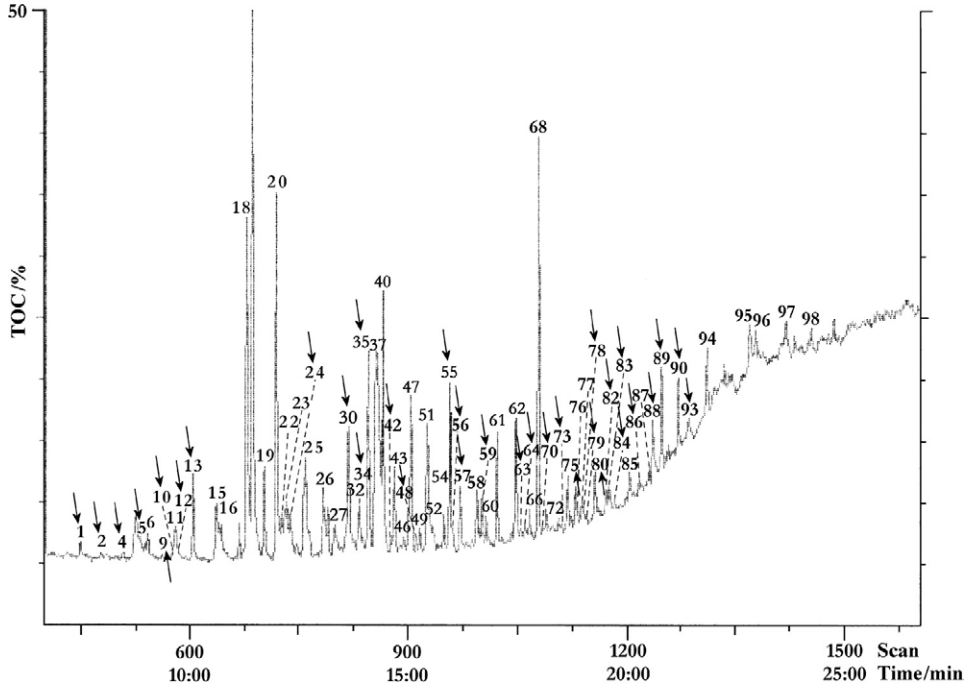


**Fig. 1.** Total ion current chromatogram of typical lime tree honey: peak identification as numbered in Table 5
Arrows indicate peaks that are not present in the volatile substance pattern of other types of honey. Only peaks belonging to honey are numbered

be considered as completely reliable markers for lime tree honey. This is especially emphasized in the context of frequent »contamination« of lime tree honey with lime tree honeydew (*1*). Absence of some volatile compounds could also be considered as a useful tool for honey characterization. Radovic *et al.* (*16*) reported that absence of 3-methyl-1-butanol (isoamyl alcohol) could confirm the authenticity of lime tree floral type (*11*), which was also the case in this study. There were some compounds identified for the first time in the studied lime tree honey samples that had not previously been reported in literature: α,α-4-trimethylbenzenemethanol (*p*-cymen-8--ol), 4-(1-methylethyl)benzenemethanol (*p*-cymen-7-ol), methylstyrene, 4-hydroxy-4-methyl-2-pentanone (diacetone alcohol), verbenol, *p*-mentha-1,5-dien-8-ol, 4-(1-methylethyl)benzaldehyde (cumin aldehyde), 4-methyl-1-(1-methylethyl)bicyclo[3.1.0]hexen-2-one (umbellulone), 4-hydroxybenzenemethanol, 2,3-dimethyltricyclo[2.2.1.02,6]heptane-3-methanol (teresantalol), 2-methyl-6-(2-propenyl)phenol (6-allyl-*o*-cresol), 5-methyl-2-(1-methylethylidene)cyclohexanone (pulegone) and 2-xylylethanol. Since they were not found in the volatile pattern of fir honeydew and sage honey samples, they could also be interesting as characteristic for lime tree honey. Identification of volatile compounds of fir honeydew honey samples was performed and shown in Table 5.

Lower number of peaks showed simpler volatile profiles compared to lime tree honey samples. In total, there were 45 peaks obtained and 37 of them were identified by HS-SPME followed by GC-MS. Volatile compounds found only in fir honeydew honey samples were acetonitrile, methyl-2-buten-1-ol, *n*-hexanol, 3-hexanol, 1-propyne, 2-furanmethanol (furfuryl alcohol), 5-methyl-2(5H)--furanone (α,β-angelica lactone), 4-methylphenol (*p*-cresol), hexadecanoic acid, and methyl ester of heptanoic acid (methylheptanoate). These volatile compounds could be considered as characteristic for fir honeydew honey. In their study, Verzera *et al.* (*17*) identified *n*-hexanol and hexadecanoic acid in several honeys (eucalyptus, orange, chestnut and wildflower), but the presence of a small amount of honeydew in nectar honeys could be of common occurrence (*1,20*). Alissandrakis *et al.* (*31*) found that 4-methoxybenzaldehyde (*p*-anisaldehyde) was a common compound of several types of honey including fir honeydew honey, but the results of this study showed its presence both in lime and sage honey and the lack of it in fir honeydew honey. Piasenzotto *et al.* (*18*) indicated borneol as lime tree honey marker, while Soria *et al.* (*19*) proposed borneol as the compound with high discrimination power for honeydew honey. They also proposed 1-(2-furanyl)ethanone (acetylfuran) as volatile compound with strong negative correlation with the presence of honeydew in honey, and at the same time a powerful discriminative tool for characterization of pure nectar honeys. In this study, these substances were identified both in lime tree and fir honey samples, so it seems that they could not be considered as reliable marker compounds for either types of honey. Inconsistencies with findings of Soria *et al.* (*19*) could arise from the fact that most honeydew honey samples studied in their research were not derived from fir honeydew (*A. alba*), but mostly from oak honeydew (*Quercus* sp.). Qualitative data evaluations following the same pattern described in previous sections were performed to characterize volatile profile of sage honey samples. Sage honey volatile profile showed the lowest number of peaks (34), compared to lime tree and fir honeydew honey samples, as shown in Table 5. Volatile compounds identified only in sage honey samples were tetrahydro-2,2,5,5-tetramethylfuran, lilac aldehyde, 3-hexenyl ester of butanoic acid, 2-methylbenzene,

Table 5. Tentative identification of volatile compounds in three unifloral honeys by HS-SPME followed by GC-MS

| Peak No. | Scan | RI | Reference | Identification | Honey | | |
|---|---|---|---|---|---|---|---|
| | | | | | **LT** | **FH** | **S** |
| 1 | 449 | 1231 | (*16–18*) | *trans*-2-caren-4-ol | X | | |
| 2 | 477 | 1256 | (*16–18*) | terpinene (isomer not identified) | X | | |
| 3 | 483 | 1261 | | n.i. fr. 37 (100), 55 (55), 67 (68), 77 (10), 91 (30), 105 (15), 117 (10) | X | | X |
| 4 | 507 | 1282 | (*31*) | 1-methyl-4-(1-methylethyl)benzene | X | | |
| 5 | 538 | 1310 | | n.i. fr. 39 (459, 79 (50), 91 (68), 105 (100), 120 (68), 132 (70) | X | | |
| 6 | 542 | 1314 | | n.i. fr. 31 (30), 43 (100), 88 (18) | X | | X |
| 7 | 542 | 1315 | | acetonitrile | | X | |
| 8 | 562 | 1334 | (*16*) | methyl-2-buten-1-ol (isomer not identified) | | X | |
| 9 | 568 | 1341 | | n.i. fr. 39 (55), 58 (33), 77 (60), 91 (100), 103 (42), 119 (15), 135 (23), 146 (15), 156 (15), 177 (30), 207 (50) | X | | |
| 10 | 575 | 1348 | | methylstyrene (isomer not identified) | X | | |
| 11 | 581 | 1353 | | n.i. fr. 30 (34), 44 (80), 73 (100), 81 (90), 93 (15), 108 (40) | X | | |
| 12 | 595 | 1367 | (*17,18,32*) | rose oxide | X | | |
| 13 | 605 | 1377 | | 4-hydroxy-4-methyl-2-pentanone | X | | |
| 14 | 618 | 1390 | (*17*) | *n*-hexanol | | X | |
| 15 | 636 | 1408 | | n.i. fr. 41 (100), 57 (60), 70 (40), 81 (33), 95 (25) | X | X | X |
| 16 | 642 | 1416 | | n.i. fr. 41 (90), 57 (100), 132 (22), 69 (16), 81 (13), 96 (11) | | X | |
| 17 | 649 | 1423 | | tetrahydro-2,2,5,5-tetramethylfuran | | | X |
| 18 | 678 | 1455 | (*16–18*) | dimethyl styrene isomer | X | X | |
| 19 | 703 | 1482 | (*15–19,32*) | 2-furanocarboxaldehyde | X | X | X |
| 20 | 719 | 1500 | (*18,19*) | 4,5,6,7-tetrahydro-3,6-dimethyl-benzofuran[a] | X | X | |
| 21 | 728 | 1511 | | n.i. fr. 71 (5), 97 (5), 135 (10), 168 (8), 193 (100), 209 (30) | | X | |
| 22 | 732 | 1515 | | n.i. fr. 41 (100), 55 (56), 67 (43), 82 (42) | X | X | |
| 23 | 740 | 1525 | (*16,19*) | 1-(2-furanyl)ethanone | X | X | |
| 24 | 749 | 1536 | | n.i. fr. 43 (90), 69 (33), 85 (40), 95 (20), 137 (100), 109 (30) | X | | |
| 25 | 758 | 1547 | (*14,16–19*) | benzaldehyde | X | X | X |
| 26 | 784 | 1577 | (*14–19*) | 2-methylpropanoic acid | X | X | X |
| 27 | 800 | 1596 | (*15,16,32*) | 5-methyl-2-furanocarboxaldehyde | X | | X |
| 28 | 808 | 1607 | (*18,19,32*) | lilac aldehyde | | | X |
| 29 | 819 | 1621 | | butanoic acid, 3-hexenyl ester (isomer not identified) | | | X |
| 30 | 819 | 1621 | (*18*) | 4-methyl-1-(1-methylethyl)-3-cylohexen-1-ol[a] | X | | |
| 31 | 821 | 1622 | | n.i. fr. 41 (98), 54 (28), 71 (100), 79 (42), 93 (25), 111 (17), 167 (10) | | X | |
| 32 | 833 | 1639 | (*18*) | butanoic acid | X | | X |
| 33 | 834 | 1639 | | 3-hexanol | | X | |
| 34 | 838 | 1645 | | n.i. fr. 39 (100), 51 (19), 67 (50), 79 (80), 94 (55), 109 (72),121 (80), 137 (28), 152 (25) | X | | |
| 35 | 846 | 1655 | | n.i. fr. 39 (30), 51 (14), 63 (10), 77 (20), 91 (35), 105 (88), 119 (40), 133 (50), 148 (100) | X | | |
| 36 | 847 | 1656 | | 1-propyne | | X | |
| 37 | 856 | 1668 | (*15–19,32*) | benzeneacetaldehyde | X | X | X |
| 38 | 860 | 1673 | (*16,17,19*) | 2-furanmethanol | | X | |
| 39 | 865 | 1679 | | 2-methylbenzene | | | X |
| 40 | 866 | 1681 | | n.i. fr. 41 (92), 57 (42), 74 (100), 91 (35), 103 (10), 135 (30) | X | | |
| 41 | 866 | 1681 | | heptanoic acid, methyl ester | | X | |
| 42 | 878 | 1697 | | verbenol (isomer not identified) | X | | |
| 43 | 882 | 1702 | (*18*) | 1-(2,4-dimethylphenyl)ethanone | X | X | |
| 44 | 883 | 1702 | | n.i. fr. 43 (50), 57 (88), 71 (60), 91 (100) | | | X |
| 45 | 894 | 1717 | | n.i. fr. 39 (68), 58 (10), 81 (10), 96 (65), 68 (100), 109 (28) 124 (20), 152 (35) | | | X |
| 46 | 896 | 1720 | (*16–19*) | borneol | X | X | |
| 47 | 905 | 1733 | | 4,7-dimethyl-benzofuran | X | X | |
| 48 | 914 | 1743 | | *p*-mentha-1,5-dien-8-ol | X | | |
| 49 | 917 | 1747 | | n.i. fr. 39 (28), 60 (100), 73 (45), 123 (20) | X | | X |
| 50 | 926 | 1761 | | n.i. fr. 43 (58), 55 (68), 67 (50), 81 (25), 93 (100), 111 (62), 117 (15), 133 (15), 155 (18) | | | X |
| 51 | 927 | 1761 | | 1-methyl-4-(1-methylethyl)benzene | X | X | |
| 52 | 936 | 1773 | (*14,18,32*) | 3-pyridine carbonitrile | X | | X |

Table 5. – continued

| Peak No. | Scan | RI | Reference | Identification | Honey | | |
|---|---|---|---|---|---|---|---|
| | | | | | **LT** | **FH** | **S** |
| 53 | 947 | 1788 | | 5-methyl-2(5H)furanone | | X | |
| 54 | 950 | 1793 | | dehydromethylene-2(3H)furanone | X | X | |
| 55 | 959 | 1804 | (*16*,*18*) | 1-(4-methylphenyl)ethanone | X | | |
| 56 | 963 | 1810 | | 4-(1-methylethyl)benzaldehyde | X | | |
| 57 | 972 | 1823 | (*18*) | α-terpinen-7-al | X | | |
| 58 | 995 | 1856 | (*14*,*17*,*18*,*32*) | hexanoic acid | X | X | X |
| 59 | 1003 | 1866 | (*17*,*32*) | α,α-4-trimethylbenzenemethanol | X | | |
| 60 | 1007 | 1873 | (*17*,*32*) | (E)-6,10-dimethyl-5,3-undecandien-2-one | X | X | X |
| 61 | 1022 | 1895 | (*14–19*,*32*) | benzenemethanol | X | X | X |
| 62 | 1048 | 1932 | (*14–19*,*32*) | benzeneethanol | X | X | X |
| 63 | 1055 | 1942 | | n.i. fr. 45 (100), 55 (51), 67 (50), 71 (45), 91 (95), 117 (55), 137 (45), 150 (90), 177 (30) | X | | |
| 64 | 1061 | 1952 | (*17*) | 4-methyl-1-(1-methylethyl)-bicyclo[3.1.0]hexen-2-one[a] | X | | |
| 65 | 1067 | 1960 | (*17*) | heptanoic acid | | | X |
| 66 | 1068 | 1961 | | 2-methyl-3-phenyl-2-propenal | X | X | |
| 67 | 1071 | 1966 | | n.i. fr. 44 (100), 54 (20), 123 (25), 69 (18), 114 (15), 138 (12), 128 (22) | | X | |
| 68 | 1080 | 1978 | | n.i. fr. 39 (47), 51 (15), 65 (23), 79 (35), 93 (100), 108 (25), 121 (22), 136 (77)[b] | X | X | X |
| 69 | 1085 | 1987 | | maltol | | | X |
| 70 | 1086 | 1989 | | n.i. 45 (68), 55 (42), 77 (42), 91 (80), 105 (38), 121 (100), 152 (40) | X | | |
| 71 | 1097 | 2004 | | n.i. 39 (75), 67 (10), 95 (45), 124 (100) | | | X |
| 72 | 1107 | 2021 | (*15*,*18*,*19*) | phenol | X | | X |
| 73 | 1111 | 2027 | | n.i. fr. 41 (35), 51 (25), 63 (12), 79 (70), 91 (100), 105 (50), 119 (25), 133 (40), 148 (75) | X | | |
| 74 | 1116 | 2034 | | 3-furanocarboxylic acid, methyl ester | | | X |
| 75 | 1119 | 2039 | | 2-xylylethanol | X | | |
| 76 | 1133 | 2059 | (*18*) | 4-methoxybenzaldehyde | X | | X |
| 77 | 1138 | 2067 | (*17*,*19*) | octanoic acid | X | X | X |
| 78 | 1141 | 2072 | | n.i. fr. 39 (48), 51 (22), 65 (25), 77 (35), 91 (100), 105 (75), 119 (42), 133 (30), 148 (78) | X | | |
| 79 | 1145 | 2078 | | n.i. fr. 38 (22), 43 (100), 55 (82), 71 (55), 83 (60), 97 (25), 109 (22), 125 (10), 153 (55), 168 (35) | X | | |
| 80 | 1156 | 2096 | | 4-hydroxybenzenemethanol | X | | |
| 81 | 1157 | 2096 | (*32*) | 4-methylphenol | | X | |
| 82 | 1171 | 2119 | (*32*) | 4-(1-methylethyl)benzenemethanol | X | | |
| 83 | 1174 | 2125 | | n.i. fr. 39 (100), 53 (19), 68 (71), 96 (85), 109 (28), 123 (15), 151 (80), 166 (50) | X | | |
| 84 | 1184 | 2140 | | teresantalol | X | | |
| 85 | 1205 | 2174 | (*14*,*16*,*19*) | nonanoic acid | X | X | |
| 86 | 1217 | 2194 | (*15*) | thymol | X | | |
| 87 | 1231 | 2217 | | 1-(2-hydroxy-5-methylphenyl)ethanone | X | X | |
| 88 | 1236 | 2226 | (*14*,*18*,*19*) | carvacrol | X | | |
| 89 | 1249 | 2247 | | 2-methyl-6-(2-propenyl)phenol | X | | |
| 90 | 1272 | 2286 | | pulegone | X | | |
| 91 | 1276 | 2293 | | n.i. fr. 44 (100), 65 (10), 77 (15), 91 (40), 103 (35), 121 (30), 146 (20) | | X | |
| 92 | 1285 | 2308 | | n.i. fr. 43 (100), 89 (35), 73 (18), 58 (15), 133 (12) | | X | |
| 93 | 1286 | 2311 | | n.i. fr. 45 (100), 55 (20), 79 (70), 91 (65), 107 (40), 133 (40), 148 (55) | X | | |
| 94 | 1311 | 2352 | (*18*) | 2-(*p*-methoxyphenyl)ethanol | X | X | |
| 95 | 1370 | 2447 | (*14*,*17*,*19*) | benzoic acid | X | X | X |
| 96 | 1378 | 2459 | | 9-octadecanoic acid, methyl ester derivative | X | X | |
| 97 | 1418 | 2499 | | n.i.[c] | X | X | X |
| 98 | 1454 | 2563 | (*18*) | benzenedicarboxylic acid derivative | X | X | |
| 99 | 1457 | 2567 | (*15*,*16*) | benzeneacetic acid | | | X |
| 100 | 1485 | 2602 | | n.i. fr. 43 (100), 54 (38), 73 (15), 89 (28), 111 (30), 115 (20), 133 (10), 173 (65), 220 (20) | X | X | X |
| 101 | 1628 | 2738 | (*17*) | hexadecanoic acid | | X | |

LT, FH and S: lime tree, fir honeydew and sage honey, respectively; n.i.: not identified; RI: retention index; [a]very similar to the compound reported in the literature reference data; [b]sabinene or β-phelandrene or 3-carene; [c]probable several volatile substances with the same RT under applied GC conditions

heptanoic acid, maltol, methyl ester of 3-furanocarboxylic acid and benzeneacetic acid. This is the first characterization of the volatile profile of sage honey reported in literature and with the exception of lilac aldehyde and heptanoic acid, their presence could be considered as characteristic for sage honey volatile profile. Namely, lilac aldehyde had previously been reported as aroma constituent of citrus and thyme honeys (*18*), and its isomers were found in several Spanish honeys (*19,20*), while heptanoic acid was identified by Verzera *et al.* (*17*) in different honeys.

## Conclusions

Comparing the results of the study of three types of honey (lime tree, fir honeydew, sage), it can be concluded that it is possible to distinguish them by characteristic compounds belonging to the sample volatile pattern. According to the available literature data, similarities between the same types of honey with different geographic origin have been found in cases of lime tree and fir honeydew honeys. On the other hand, sage honey has been found as quite different compared to the other studied honeys, as well as compared to the honeys described in the literature, which confirms the valuable contribution of the HS-SPME followed by GC-MS analyses in determination of the common characteristics of monofloral honeys.

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