Volatile profile of Spanish raw citrus honey: the best strategy for its correct labelling

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Abstract

The objective of this study was to evaluate the presence of specific volatile compounds (analyzed by SPME-GC-MS) in citrus honeys, comparing thyme and sunflower, and to correlate their abundance with the level of methyl-anthranilate (accepted as a true marker in citrus honey), for which a minimum content is mandatory in commercial transactions. Methyl-anthranilate is well correlated with certain volatile compounds such as 1-p-menthene-9-al (0.903), limonene (0.885), dill-ether (0.842) and ethyl linalool (0.832), and slightly lower with the four lilac aldehydes (0.717 to 0.764). However, the latter, together with methyl-anthranilate, are of special interest because these were found in all citrus honey samples. Consequently, the information provided by these five compounds could facilitate the unmistakable classification of this honey. Thanks to these positive results and even though that this method is not commonly practiced to objectively differentiate between monofloral honeys it can be considered an interesting analytical tool in the future.

Practical applications

A proper classification of citrus honey before entering as a raw material in the honey packaging process would ensure its correct labeling. This would benefit the consumer and the beekeepers. The results of this study intend to shed some light to help the industry achieve a correct cataloging of citrus honey by proposing an alternate technique based on the characterization of its volatile fraction.

Keywords: Citrus honey, Monofloral honey, Volatile profile, SPME-GC-MS.

1. INTRODUCTION

The beekeeping sector is conscious of the importance of marketing monofloral honey specifying its botanical origin (B.O.E., 2018). This improves producers' profit margins since consumers are willing to pay more for honey with specific sensory nuances. Among monoflorals, the citrus honey is highly valued due to its delicate flavour evokes the orange blossom.

A honey is classified as monofloral from a specific botanical origin, when a certain percentage of pollen of this type of plant is present (Escriche, Sobrino-Gregorio, Conchado & Juan-Borrás, 2017). This is because the bees are impregnated with pollen when they visit the plants to collect the nectar or the sweet secretions of insects or plants. This honey cataloging procedure (by optic microscope) is very complex, mainly because it requires highly expert technicians in the identification and quantification of pollens from different botanical species (Tanleque-Alberto, Juan-Borras & Escriche, 2019). Each type of monofloral honey requires a different percentage of pollen from a specific specie (in relation to the other pollens present in the sample). However, in citrus honey, as a result of the cultivation of hybrid trees, that generate small amount of pollen, there is an added problem for its cataloguing due to the low presence of citrus pollen.

For this reason, the application of alternate techniques to the traditional pollen analysis in the classification of monofloral honeys is a necessity for the beekeeping sector.

Among them, it is worth highlighting the analysis of the volatile fraction by chromatography, as it is closely related to the intrinsic flavor that the consumer perceives when eating each type of monofloral honey. Among these volatile compounds, methyl anthranilate is especially important in citrus honey since it is only present in the orange blossom nectar (Juan-Borrás et al., 2015).

The objective of this study was to evaluate the presence of specific volatile compounds in citrus honeys (comparing thyme and sunflower) and to correlate their abundance with the methyl anthranilate level.

2. MATERIALS AND METHODS

2.1. Honey samples

A total of 50 honey samples were analyzed, 25 from citrus and other 25 with a predominant abundance of pollen of other varieties. All these were collected in 2021 and provided by Spanish beekeepers, either directly or through *Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente* (Ministry of Spanish Agriculture and Fishing, Food and Environment), thanks to an agreement with this Ministry with the laboratory where the present study was conducted LABMIEL: *Laboratorio de la Miel y los Productos Apícolas del Instituto de Ingeniería de Alimentos para el Desarrollo. Universitat Politecnica de Valencia, España* (Laboratory of Honey and Bee Products placed at the Institute of Food Engineering for Development, Universitat Politècnica de València., Spain) (B.O.E, 2018). The samples were considered as belonging to these botanical varieties taking into account the information provided by the pollen analysis performed following the International Commission for Bee Botany recommendations (Louveaux, Maurizio, & Vorwohl, 1978; Persano-Oddo & Piro, 2004). Pollen grains were

identified considering the reported by Orantes-Bermejo & Gómez-Pajuelo in 2009 and a general palynological database (Palynological Database online, 2018).

Based on the limits agreed by the commercial transactions (since no official values have been established), in the present work the criterion was that a honey was considered as monofloral from citrus if the pollen from *Citrus sp*. was not lower than 10%. In addition, the organoleptic characteristics related to smell, taste and appearance were also taken into account in all cases. The other samples were selected for this study due to their special abundance in pollen from other botanical varieties, specifically, thyme (*Thymus sp.)* and sunflower (*Helianthus annuus*).

A new software developed by the Institute of Control Systems and Industrial Computing (AI2) at the Universitat Politecnica de Valencia was used to take the pictures from the slides and to count and classify these honey pollens. Figure 1 shows examples of different photomicrographs corresponding to them. The identification of the pollen morphologies is an indispensable step to confirm the monoflorality of the samples. Table S1 (Supplementary material) shows the pollen spectrum of each sample.

2.2. Volatile compounds analysis

Volatile compounds of the honey samples were extracted by solid-phase micro-extraction (SPME) and analyzed using gas chromatography/mass spectrometry (GC/MS) 8 g of honey were weighed into 20 mL screw cap vials equipped with PTFE silicon septum and dissolved in 1 mL of bidistilled water plus 2 mL of saturated NaCl solution, 1g of glass beads were added to facilitate homogenization. The vials were vortexed for 2 minutes until complete homogenization of the sample. A DVB/CAR/PDMS (divinyl benzene/carboxen/polydimethylsiloxane, 50/30 μm) fiber was used to trap the honey volatile compounds exposed to the headspace of the sample for 30 minutes, maintaining all the time the vial on a heating platform agitation at 50 °C, 250 rpm. Then, the fiber was

inserted into the injection port of the GC/MS System and desorbed in the GC injector for 30 min at 230 °C.

The analysis of volatile compounds was performed using an Agilent Intuvo 9000 gas chromatograph coupled to an Agilent 7000 Series GC/TQ triple quadrupole detector equipped with an electron ionization source at 70 eV. The chromatographic separation was carried out in a DB WAX column (Agilent, 30m x 0.25mm x 0.25µm) with helium as the carrier gas (at a flow rate of 1.0 mL/min). The temperature of the oven was programmed starting at 35 °C for 3 minutes, it rose to 215 °C at 5 °C/min and finally rose to 250 °C at 30 °C/min and remained at this temperature for 6 minutes. The mass spectra were acquired in the total ion chromatography (TIC) mode with a mass range of m/z 40-280. Data acquisition and analysis were performed using the MassHunter Workstation software (Unknow analysis).

The identification of each volatile compound was accomplished by comparing their mass spectra, retention times and linear retention indices (LRI) with those obtained from authentic standards (Sigma-Aldrich, St Louis, MO; Acros Organics, Geel, Belgium and Fluka Buchs, Switzerland). For those compounds for which this was not possible due to not having authentic standards, the tentative identification was carried out by comparing their mass spectra with the spectral data from the National Institute of Standards and Technology 2002 library (always considering a match factor ≥80%), as well as the linear retention indices and data published in the literature (Shimoda, Wu, & Osajima, 1996; Bianchi, Careri, Mangia, & Musci, 2007; Goodner, K.L., 2008). The linear retention indices of all the compounds were obtained by injecting a mixture of a homogenous series of alkanes (C8–C20, Fluka Buchs, Schwiez, Switzerland) under the same chromatographic conditions as described above for the samples. To estimate the abundance of each compound, deconvolution base peak area was considered (average

value for two replicates) similar as reported by Verzera, Tripodi, Condurso, Dima, & Marra (2014).

2.3. Statistical analysis

Bivariate Pearson correlations were obtained (α=0.05) to measure the direction and strength of the linear relationships between pairs of variables using the XLSTAT statistical and data analysis solution Addinsoft (2021), New York, USA. The data were also analyzed by using a PCA multivariate technique (Principal Component Analysis) applying the software Unscrambler X.10. The variables were centered and weighted.

3. RESULTS AND DISCUSSION

A typical volatile profile chromatogram of the three monofloral honey studied is shown in Figure 2. The difference in the chromatogram plot for each variety, illustrates that their volatile fraction could contain enough information useful for their differentiation. Therefore, it makes sense to analyze in depth the conduct of the compounds present in the respective volatile fraction.

In the volatile fraction of the three types of honey, 100 volatile compounds were identified, which are shown in Table 1, together with their linear retention indices (LRI) calculated and the ANOVA results (F-ratio and significant differences) obtained for the factor “type of monofloral”. The data (maximum, minimum, average, and standard deviation) are expressed as deconvolution base peak areas. One third of the compounds did not show significant differences among the three types of honey and therefore they are not relevant for their differentiation.

Among the compounds found in citrus honey, the methyl anthranilate and certain linalool derivatives such as lilac aldehydes, linalool oxides, dill ether, among others, stand out for their presence or significant abundance in comparison with the other honeys (thyme and

149 sunflower). These findings are in line with what was reported by different authors
150 (Alissandrakis, Tarantilis, Harizanis & Polissiou, 2007; Verzera, Tripodi, Condurso,
151 Dima, & Marra, 2014); Seraglio, Schulz, Brugnerotto, Silva, Gonzaga, Fett & Costa,
152 2021). They affirmed that due to the relative low level or even absence of these
153 compounds in other types of monofloral honeys, they can be considered as powerful
154 markers for citrus honey. Nevertheless, among all these compounds, special attention
155 must be paid to methyl anthranilate, not only because it is a specific compound in citrus
156 blossom nectar (its aroma is characteristic of this type of flower, ISO 5496, 2006), but
157 also because of its commercial value. In fact, in the commercial transactions a content of
158 at least 2 mg/kg of methyl anthranilate together with a minimum citrus pollen content
159 (between 10 and 20%) is mandatory to be considered a true citrus honey (Juan-Borrás,
160 Periche, Domenech, & Escriche, 2015).

161 With the aim of ascertaining the possible linear dependence between the citrus volatile
162 compounds here identified, the Pearson correlation coefficients were calculated for each
163 pair of variables (Table 2). The closer to +1 or -1 the strength of the linear relationship is
164 higher. Methyl-anthranilate correlates (P-values below 0.05) the best with 1-p-menthene-
165 9-al (0.903) and limonene (0.885), dill ether (0.842) and ethyl linalool (0.832). The
166 positive signs in all cases denote those large values of methyl-anthranilate are associated
167 with large values of these compounds. Important although slightly lower correlations
168 were found between methyl-anthranilate and the four lilac aldehydes (0.717, 0.764, 0.732
169 and 0.751, respectively). However, these four compounds are of particular interest
170 because they were identified in all honey citrus samples analyzed, and this was not the
171 case for the other compounds better correlated with methyl-anthranilate. Therefore, the
172 constant presence all together of these five compounds (methyl anthranilate and lilac

aldehydes) and their correlation occurring only in citrus honey facilitates the unmistakable classification of this type of honey and its correct labelling.

To have a more encompassing vision and to evaluate from a descriptive point of view the global effect of the type of monofloral honey on the volatile profile, a principal component analysis (PCA) was performed. Figure 3 shows the PCA plot of scores obtained, when proximity between samples suggests similarity on their volatile compounds' behavior. Two principal components explained 67% of the variations in the data set. PC1 (38%) and PC2 (29%). The first principal component clearly differentiates citrus honey (right quadrant) from sunflower (upper left quadrant) and thyme honey (bottom left quadrant). This is the case of the 25 honey samples classified as citrus that are completely differentiated from the rest. The second principal component differentiates sunflower honey quite well since it is observed some samples displacement, where a few thyme honeys are located in the sunflower zone.

The correlations between the variables (volatile compounds) and the factors after varimax rotation have proven that certain volatile compounds are associated with the principal components. Thus, the higher the value of this correlation, the greater the link with the corresponding components. Figure 4 shows the loading plot where this association can be observed. The compounds that most correlate with PC1 positive, (where citrus honey is placed) were the four lilac aldehydes (A, B, C, and D) and the methyl anthranilate. In this type of honey dill ether; 1-p-menthene-9-al; trans linalool oxide and limonene were also important.

Jasmone, acetophenone, eugenol, thymol, were found to be highly correlated to thyme since they have only been detected in this variety. Other compounds (butanoic acid; butanoic acid, 2-methyl; hexanoic acid, 4-hexenoic acid and octanal) were also correlated

with thyme honey because they were especially abundant in this variety of honey, however, they were also sometimes identified in the samples of sunflower honeys.

In sunflower honey, the correlation was more remarkable with 1-hexanol; hotrienol; heptanoic acid; hexanal; linalool 1-hexanol-4 methyl and coumarin.

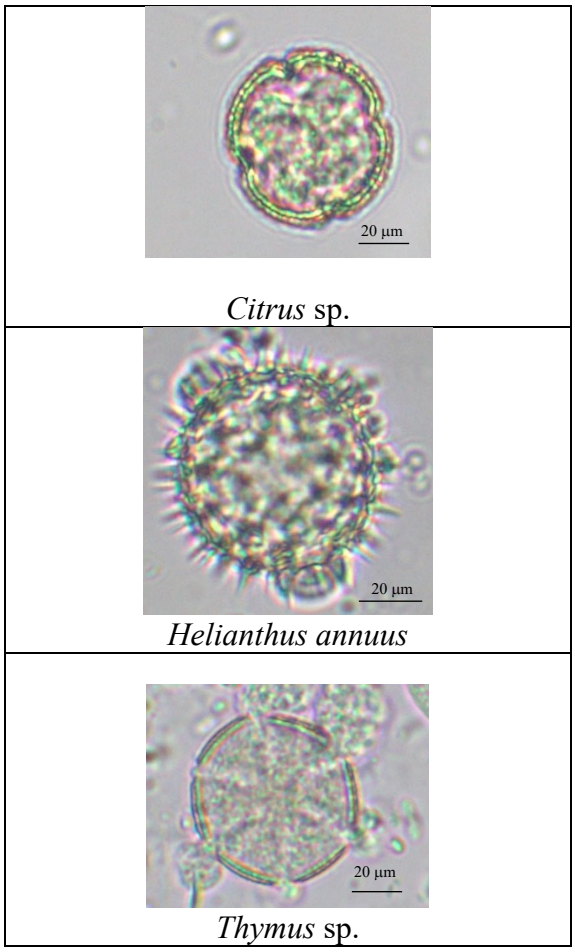
Some of these compounds have been reported by other authors who had used the SPME procedure for the extraction of the volatile fraction (Karabagias, Nikolaou & Karabagias, 2019; Machado, Miguel, Vilas-Boas & Figueiredo, 2020).

CONCLUSION

The results of this study show that the volatile fraction could contain potential useful information to objectively differentiate between monofloral honeys. The combination of the SPME-GC-MS technique with multivariate analysis is becoming an excellent tool to achieve this goal. This statement is more evident in the case of citrus honey since it contains specific volatile compounds such as methyl anthranilate and other linalool derivatives like the lilac aldehydes that are always present in this type of honey.

Consequently, the information provided, not only by methyl-anthranilate (minimum content mandatory in commercial transactions), but also of these other compounds may support the unequivocal cataloging of this honey and therefore the correct information the beekeeping sector and consumer are receiving.

This technique is not applied routinely in the classification of monofloral honeys to date; however, as the results in this research have shown, this could be considered a promising objective method for this purpose in the future.



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221 **Figure 1.** Photomicrographs of the main pollen identified in the three monofloral honey

222 samples at 400 magnifications in differential interference contrast (DIC).

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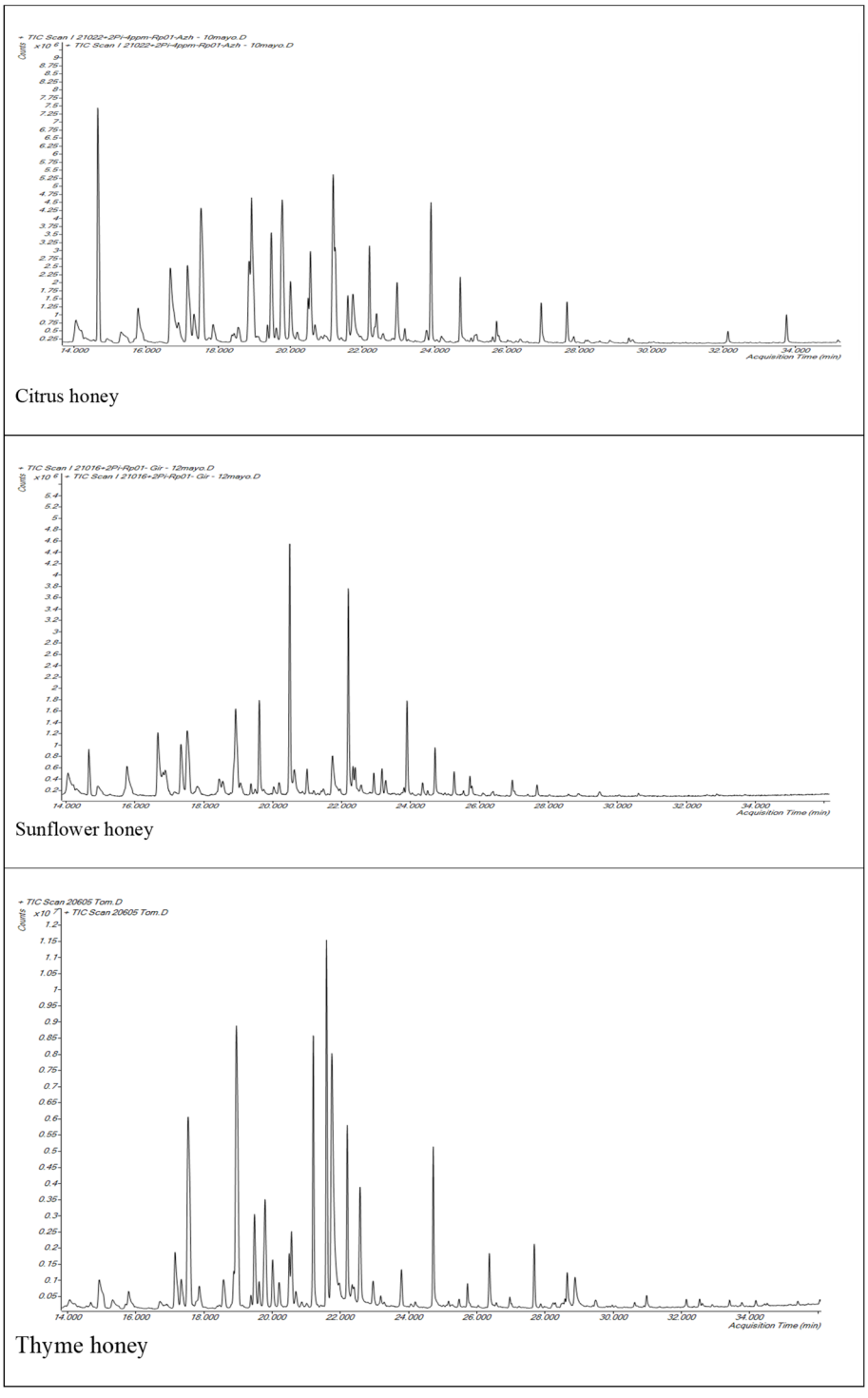

Figure 2. Typical GC-MS volatile profile chromatograms of the monofloral honeys studied.

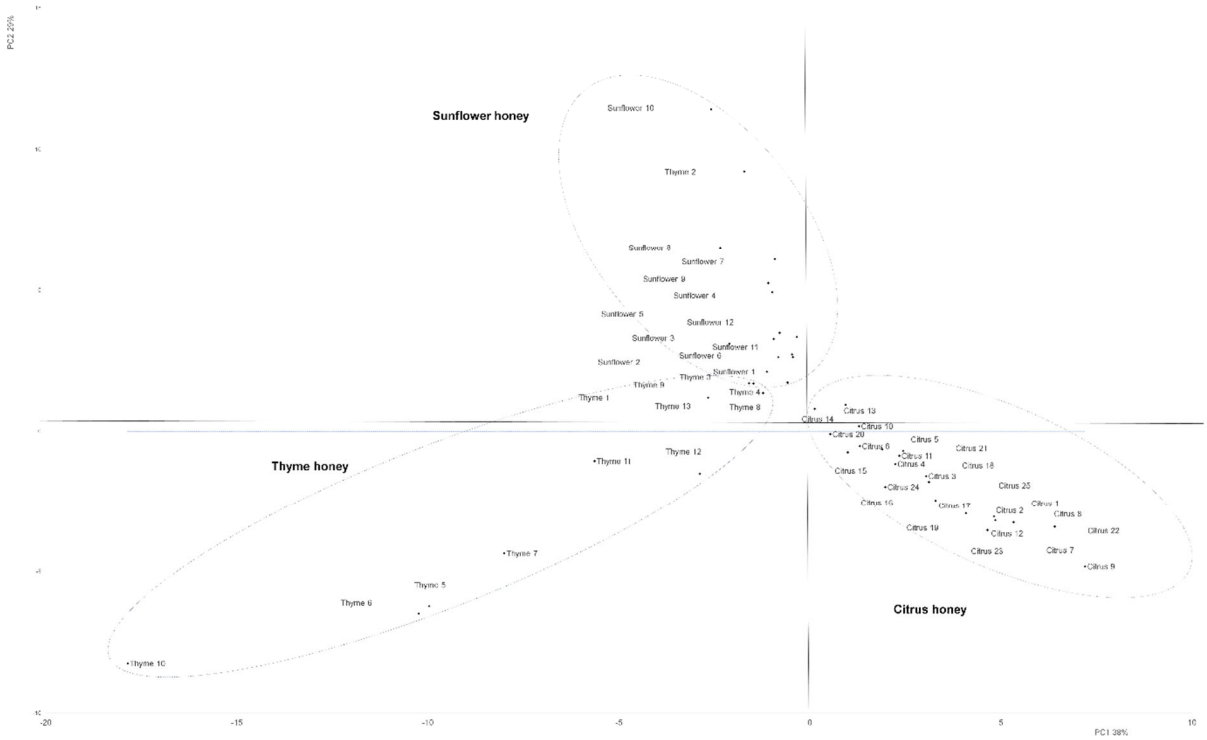

Figure 3. PCA score (three different types of honey) plot of the first two principal components. The dots indicate the botanical species of the most abundant pollen in the sample: *Citrus* sp. (citrus honey); *Helianthus* sp. (sunflower honey); *Thymus* sp (Thyme honey).

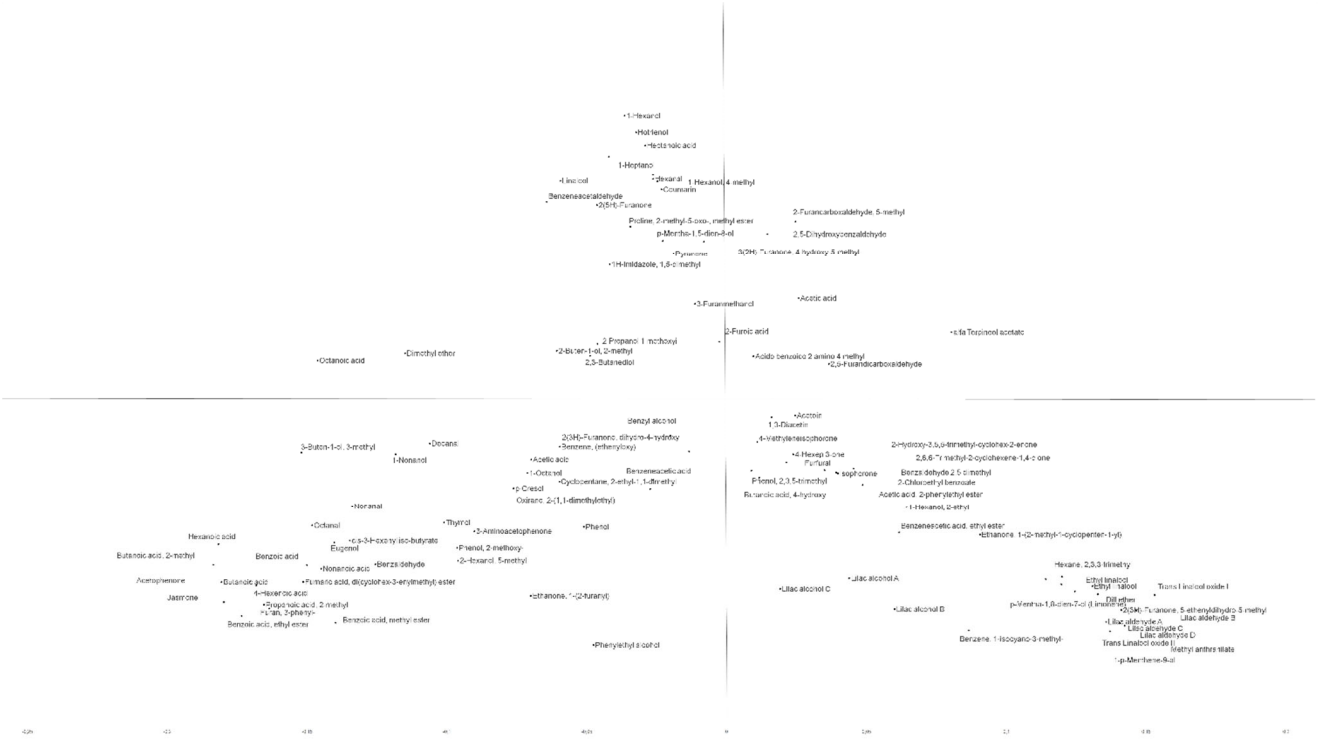

Figure 4. PCA loading (volatile compounds) plot of the first two principal components.

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263 **Table 1.** Volatile compounds and their linear retention indices (LRI) in citrus, sunflower and thyme honeys. The data (maximum, minimum,
264 average and standard deviation) are expressed as deconvolution base peak multiply by 10^6. ANOVA results (F-ratio and significant differences)
265 obtained for the factor "type of monofloral".
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		Citrus honey		Sunflower honey		Thyme honey		ANOVA F-ratio
VOLATILE COMPOUNDS	LRI[a]	Min-max	Mean (SD)	Min-max	Mean (SD)	min-max	Mean (SD)	
Hexanal	1170.1	nd	nd	nd-230	35(64)	nd-49	4(14)	5**
Oxirane, 2 (1,1-dimethylethyl)	1220.6	nd-49	11(15)	nd-9	4(3)	nd-32	11(10)	ns
3-Buten-1-ol, 3-methyl	1259.6	nd-10	0.5(2.0)	nd-13	5(4)	nd-17	7(6)	14***
Octanal	1285.0	nd	nd	nd-12	3(4)	nd-87	10(25)	ns
Acetoin	1289.9	nd-4	1(1)	nd-5	1(2)	nd-2	0.3(0.5)	ns
2-Buten-1-ol, 2-methyl	1330.8	nd-11	2(4)	nd-15	5(4)	nd-15	4(4)	3*
2-Hexanol, 5-methyl	1332.8	nd-5	0.7(1.4)	nd	nd	nd-18	1(5)	ns
1-Hexanol	1363.0	nd-4	1(1)	21-575	135(148)	nd-487	60(131)	8**
Nonanal	1394.3	1(24)	6(5)	nd-27	13(9)	nd-129	16(37)	ns
Hexane, 2,3,3-trimethyl	1431.8	nd-10	4(2)	nd	nd	nd	nd	11**
1-Hexanol, 4-methyl	1439.7	nd	nd	nd-13	2(4)	nd-7	1(18)	ns
Trans Linalool oxide I	1449.2	8-41	22(9)	nd-10	6(3)	nd-2	6(5)	27***
cis-Linaloloxide	1449.0	nd	nd	nd	nd	nd-5	0.7(2)	4**
Acetic acid	1456.8	nd-0.3	0.01(0.06)	nd	nd	nd-10	2(3)	5*
Furfural	1464.3	3-21	8(5)	nd-16	4(6)	nd-32	5(9)	ns
1H-Imidazole, 1,5-dimethyl-	1464.2	nd	nd	nd-29	7(1)	nd-13	3(4)	8**
1-Heptanol	1464.4	nd	nd	nd-200	24(59)	nd-103	10(3)	ns
Trans Linalool oxide II	1477.1	2-9	5(2)	nd-3	1.5(1.2)	nd-5	1.8(1.5)	17***
1-Hexanol, 2-ethyl-	1497.4	nd-2	0.3(0.7)	nd	nd	nd	nd	ns
Decanal	1500.3	nd-13	0.6 (2.7)	nd-7	2(18)	nd-13	2(4)	ns

Ethanone, 1-(2-furanyl)	1505.2	nd-3	0.9(1.1)	nd-1	0.2(0.4)	nd-4	1.6(1.3)	6**
Dill ether	1517.3	2-43	10(10)	nd	nd	nd	nd	13***
Benzaldehyde	1521.0	nd-29	7(7)	nd-9	4(3)	0.8-50	15(16)	5*
2,5-Dihydroxybenzaldehyde	1538.6	nd-4	1.1(1.2)	nd-3	1.9(0.9)	nd-3	1(1)	ns
Lilac aldehyde A	1543.2	6-144	40(29)	0-5	2.8(1.6)	nd-18	5(6)	18***
2,3-Butanediol	1548.8	nd-8	2(2)	nd-46	8(14)	1.3(20)	5(5)	ns
Linalool	1554.5	nd	nd	nd-65	16(19)	nd-25	8(7)	10***
Lilac aldehyde B	1556.3	3-147	47(30)	nd	nd	nd-12	2(4)	29***
Lilac aldehyde C	1565.4	2-93	26(19)	nd-3	1.3-1.4	nd-10	2(3)	19***
Dimethyl ether	1566.1	nd	nd	nd-8	1.2(3)	nd-12	2(3)	ns
1-Octanol	1567.1	nd	nd	nd	nd	nd-5	0.8(1.4)	6***
Propanoic acid, 2-methyl	1573.6	nd	nd	nd	nd	nd-4	1.2(2.1)	8***
2-Furancarboxaldehyde, 5-methyl-	1574.1	nd-3	0.8(0.8)	nd-6	1.7(2.0)	nd-3	0.5(0.9)	ns
2-Propanol, 1-methoxy	1585.7	nd-19	4(5)	nd-106	21(36)	nd-107	13(11)	3*
Lilac aldehyde D	1588.6	4-91	29(19)	0-3	1.5(1.3)	nd-12	3(4)	24***
Ethanone, 1-(2-methyl-1-cyclopenten-1-yl)-	1593.1	nd-7	1.1(1.9)	nd	nd	nd	nd	4*
Isophorone	1594.0	nd-137	9(27)	nd	nd	nd-3	0.6(1.1)	ns
Hotrienol	1615.9	nd	nd	nd-2	2.7 (0.6)	nd-1.3	0.2(0.3)	14***
Benzoic acid, methyl ester	1620.9	nd-0.3	0.03(0.09)	nd	nd	nd-0.8	0.13(0.23)	4*
Butanoic acid, 4-hydroxy-	1626.4	nd-0.2	0.02(0.07)	nd	nd	nd-0.2	0.02(0.06)	ns
Butanoic acid	1632.9	nd-1.1	0.2(0.4)	nd-20	4(5)	1.3-199	67(74)	15***
Benzeneacetaldehyde	1639.8	4-25	11(6)	3-110	29(26)	5-67	24(18)	6**
Acetophenone	1649.2	nd	nd	nd	nd	4-4	1.3(1.4)	16***
2-Hydroxy-3,5,5-trimethyl-cyclohex-2-enone	1665.2	nd-10	1(3)	nd	nd	nd	nd	ns
3-Furanmethanol	1665.9	nd-9	2(3)	nd-8	2(3)	nd-9	2(3)	ns
Benzoic acid, ethyl ester	1666.6	nd-0.8	0.06(0.20)	nd-6	nd	nd-4	1.0(1.3)	9***
Cyclopentane, 2-ethyl-1,1-dimethyl	1668.0	nd-2	0.08(0.4)	nd	nd	nd-1.4	0.2(0.5)	ns
2(3H)-Furanone, 5-ethenyldihydro-5-methyl-	1668.6	nd-5	2(1)	nd	nd	nd	nd	35**

1-Nonanol	1668.6	nd	nd	nd-3	0.5(1.1)	nd-6	2(2)	10***
Acetic acid	1675.4	nd-0.3	0.01(0.06)	nd	nd	nd-10	2(3)	5*
Butanoic acid, 2-methyl-	1676.1	nd-2	0.4(0.7)	nd-10	4(3)	1.2-69	20(23)	12***
2,6,6-Trimethyl-2-cyclohexene-1,4-dione	1692.8	nd-23	3(5)	nd-0.5	0.1(0.2)	nd-5	1.5(1.6)	ns
1-p-Menthene-9-al	1693.7	nd-2	6(4)	nd	nd	nd	nd	22**
Pentanoic acid, anhydride	1700.0	nd	nd	nd	nd	nd-2	0.7(0.7)	3*
alfa Terpineol acetate	1702.9	nd-2.8	0.9(0.8)	nd-23	1.1(0.6)	nd-1.3	0.5(0.4)	ns
Lilac alcohol A	1729.4	nd-3	0.7(0.9)	nd	nd	nd-3	0.4(0.9)	3*
4-Methyleneisophorone	1730.9	nd-43	4(9)	nd	nd	0-7	2(2)	ns
p-Mentha-1,5-dien-8-ol	1731.2	nd	nd	nd-0.6	0.2(0.2)	nd-0.9	0.01(0.2)	16***
Pentanoic acid	1743.1	nd	nd	nd	nd	nd-2	0.2(0.5)	~~4*~~
Lilac alcohol B	1749.4	nd-0.5	0.04(0.20)	nd	nd	nd-2	0.1(0.5)	ns
2(5H)-Furanone	1751.5	nd-0.2	0.1(0.4)	nd-1.1	0.2(0.4)	nd-0.1	0.2(0.3)	4*
1-Decanol	1770.8	nd	nd	nd	nd	nd-0.6	0.08(0.2)	4**
Proline, 2-methyl-5-oxo-, methyl ester	1771.9	nd	nd	nd-0.9	0.2(0.4)	nd-0.5	0.09(0.2)	6**
Acido benzoico 2 amino 4 methyl	1773.3	4-18	12(4)	11-20	14(3)	4-20	12(4)	ns
Benzeneacetic acid, ethyl ester	1787.7	nd-0.9	0.2(0.3)	nd	nd	nd-0.2	0.02(0.06)	5***
Lilac alcohol C	1789.8	nd-3	1.1(1.2)	nd	nd	nd-2	0.3(0.8)	6**
Benzaldehyde 2,5 dimethyl	1794.3	nd-0.9	0.13(0.30)	nd	nd	nd-0.6	0.05(0.16)	ns
Acetic acid, 2-phenylethyl ester	1817.3	nd-0.12	0.16(0.40)	nd	nd	nd	nd	ns
Hexanoic acid	1851.2	nd-3	0.6(0.9)	1-9	4(3)	1-60	12(20)	10***
Furan, 3-phenyl-	1853.0	nd-0.14	0.01(0.04)	nd	nd	nd-0.7	0.13(0.2)	7**
Phenol, 2-methoxy-	1861.7	nd	nd	nd	nd	nd-2	0.9(0.7)	~~5**~~
Benzyl alcohol	1880.8	nd-7	1.3(2.0)	nd-3	1.3(0.7)	0.4-2.5	1.3(0.7)	ns
Benzoic acid, m-hydroxyphenyl ester	1894.2	nd	nd	nd	nd	nd-3	0.4(0.9)	3*
Phenylethyl alcohol	1916.0	0.6(13.0)	6(4)	1.3(5.4)	3(1)	1(15)	6(4)	4*
Benzene, 1-isocyano-3-methyl-	1925.6	1.2(1.2)	0.4(0.3)	nd	nd	nd-0.3	0.09(0.13)	25***
p-mentha-1-en-9-ol	1943.8	nd-2.4	0.6-0.7	nd	nd	nd	nd	10***

Jasmone	1947.1	nd	nd	nd	nd	nd-3	0.6(0.8)	9***
cis-3-Hexenyl iso-butyrate	1955.8	nd	nd	nd	nd	nd-0.4	0.15(0.2)	11***
Heptanoic acid	1955.8	nd	nd	nd-3	2.5(0.9)	nd-2	0.15(0.6)	4*
4-Hexenoic acid	1959.0	nd	nd	nd-4	0.9(1.2)	nd-61	13(20)	8**
2,5-Furandicarboxaldehyde	1963.4	0.5-17.0	3(3)	0.7-11.0	3(3)	nd-5	2(2)	ns
p-Mentha-1,8-dien-7ol (Limonene)	1976.1	1-3	1.3(0.9)	nd	nd	nd	nd	8***
2-Furoic acid		nd	nd	nd-2	0.8(0.5)	nd-0.4	0.6(0.2)	3*
Phenol	1950.0	nd-1.2	0.2(0.3)	0-0.4	0.15(0.16)	nd-0.7	0.3(0.2)	ns
Octanoic acid	2008.0	nd-0,6	0.09(0.2)	nd-2	0.9(0.7)	nd-3	0.7(0.7)	12***
1,3-Diacetin		nd-2	2(6)	nd-4	2.0(1.5)	nd-5	1.3(1.8)	ns
p-Cresol		nd-0.1	0.08(0.03)	nd	nd	nd-1.1	3(4)	9***
3(2H)-Furanone, 4-hydroxy-5-methyl-	2037.0	nd-0.7	0.08(0.18)	nd	nd	nd-0.9	0.14(0.30)	ns
Ethyl linalool	2063.2	1-3	0.8(0.8)	nd	nd	nd	nd	12***
Nonanoic acid	2096.0	nd-0.2	0.01(0.05)	nd	nd	nd-4	0.40(1.15)	3*
Eugenol	2115.0	nd	nd	nd	nd	nd-0.9	0.3(0.4)	13***
Thymol	2136.1	nd	nd	nd	nd	nd-0.3	0.6(1.1)	7**
3-Aminoacetophenone	2165.2	nd	nd	nd	nd	nd-1.5	0.1(0.4)	4*
Methyl anthranilate	2230.0	2-13	5(3)	nd	nd	nd	nd	25***
Pyranone	>2230	nd-19	2(4)	nd-21	5(8)	nd-29	5(9)	ns
Fumaric acid, di(cyclohex-3-enylmethyl) ester	>2230	nd	nd	nd	nd	nd-1.3	0.2(0.4)	5*
Phenol, 2,3,5-trimethyl	>2230	nd-7	0.6(1.4)	nd	nd	nd-2	0.2(0.6)	ns
Coumarin	>2230	nd	nd	nd-0.6	0.12(0.20)	nd-1.1	0.13(0.30)	3*
Benzoic acid	>2230	nd-0.14	0.05(0.30)	nd-3	0.8(0.9)	nd-19	6(7)	14***
2(3H)-Furanone, dihydro-4-hydroxy-	>2230	nd-0.7	0.08(0.20)	nd	nd	nd-1.3	0.14(0.30)	ns

[a] LRI= Linear retention indices calculated on a DB WAX column.
ns: Non significant differences ; p*<0.05; p**<0.

Table 2. Correlation matrix (Pearson correlation coefficients) between the most important volatile compounds found in citrus honey.

Variables	Methyl anth.	Trans Lin I.	Dill ether	Lilac ald. A	Lilac ald. B	Lilac ald. C	Lilac ald. D	1-p-Menth	Limon.	Trans Lin II	Ethyl linal.
Methyl anthranilate	1	0.774	0.842	0.717	0.764	0.732	0.751	0.903	0.885	0.723	0.832
Trans Linalool oxideI	0.774	1	0.824	0.849	0.877	0.852	0.860	0.881	0.756	0.944	0.688
Dill ether	0.842	0.824	1	0.782	0.798	0.791	0.779	0.938	0.878	0.777	0.706
Lilac aldehyde A	0.717	0.849	0.782	1	0.982	0.995	0.994	0.793	0.642	0.800	0.563
Lilac aldehyde B	0.764	0.877	0.798	0.982	1	0.979	0.989	0.833	0.676	0.822	0.597
Lilac aldehyde C	0.732	0.852	0.791	0.995	0.979	1	0.990	0.805	0.659	0.809	0.574
Lilac aldehyde D	0.751	0.860	0.779	0.994	0.986	0.990	1	0.807	0.670	0.804	0.604
1-p-Menthene-9-al	0.903	0.881	0.938	0.793	0.833	0.805	0.807	1	0.903	0.833	0.762
Limonene	0.885	0.756	0.878	0.642	0.676	0.659	0.670	0.903	1	0.715	0.808
Trans Linalool oxideII	0.723	0.944	0.777	0.800	0.822	0.809	0.804	0.833	0.715	1	0.655
Ethyl linalool	0.832	0.688	0.706	0.563	0.597	0.574	0.604	0.762	0.808	0.655	1

P-value below 0.05

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