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Volatile profile in the accurate labelling of monofloral honey. The case of lavender and thyme honey

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ABSTRACT

The proliferation of hybrid plant varieties without pollen, such as lavender, has complicated the classification of specific types of honey. This study evaluated the correlation between the proclaimed type of monofloral honey (lavender or thyme) as appears on the label with the actual percentage of pollen. In addition, physicochemical parameters, colour, olfato-gustatory profile, and volatile compounds were tested. All the samples labelled as lavender were wrongly classified according to the usual commercial criteria (minimum 10% of pollen *Lavandula* spp.). In the case of lavender honey, there was significant agreement between commercial labelling and classification through organoleptic perception (81.8%), and above all between the commercial labelling and the volatile compounds (90.9%). For thyme honey, agreement for both parameters was 90.0%. These results offer compelling evidence that the volatile compounds are useful for the classification of lavender honey with low levels of pollen since this technique agrees well with the organoleptic analysis.

Keywords: accurate labelling; lavender honey classification; thyme honey; volatile compounds; GC-MS

1. Introduction

Thyme and lavender honey, although limited in production, are highly appreciated due to their organoleptic characteristics. Both, thyme (*Thymus* spp.) and lavender (*Lavandula* spp.) plants belong to the botanical family Labiatae and are part of the autochthonous Mediterranean vegetation, sharing environmental characteristics and botanical habitat and pollinating agents. These types of honey have a characteristic sweet flavour with sour notes, but may have salty notes when harvested late and/or contain some honeydew secretions. Thyme honey has an intense persistent aroma and its colour ranges from a very light amber to amber with red highlights. Lavender honey has a distinctive aroma, which is very characteristic of the lavender plant. Its colour varies from amber to very light amber, being clearer when purer, and darker due to a higher content of oak honeydew (Mateu, 2002).

The monoflorality of honey is determined by the botanical species that bees visit to obtain the nectar from flowers or the secretions of plants. Honey can be classified as belonging to a specific botanical origin when a certain percentage of pollen of this botanical species is present. The required percentage varies depending on the botanical species in question; for example, ranges between 10-20% for orange blossom honey or 70-90% for eucalyptus honey (Persano-Oddo & Piro, 2004; Juan-Borrás, Domenech, Conchado, & Escriche, 2015). However, pollen may be under represented, which causes problems in the botanical classification of honey (Persano-Oddo & Piro, 2004; Juan-Borrás et al., 2015, ANIPAM, 2008; The Apis Information Resource Center website, 2016). This problem has been observed in citrus honey because citrus trees sometimes

produce nectar before the anther produces pollen, or the nectar comes from sterile hybrid varieties of citrus trees which are characterized by their small amounts of pollen (Juan-Borrás, Domenech, & Escriche, 2015).

In the case of lavender honey, the problem is similar because the percentage of pollen is usually very low or even non-existent. This is because in recent years the lavender crop has consisted of hybrid varieties without pollen, which therefore is not present in the nectar nor the honey (Persano-Oddo & Piro, 2004). This problem for the honey sector has been noticed by the laboratories which carry out the botanical classification of honey.

For this reason, it is recommendable to complement the results of pollen analysis with other determination techniques, such as physicochemical (Bogdanov, Ruoff, & Persano-Oddo, 2004; Kádár, Juan-Borrás, Carot, Domenech, & Escriche, 2011), organoleptic (González-Viñas, Moya, & Cabezudo, 2003; Castro-Vázquez, Díaz-Maroto, González-Viñas, & Pérez-Coello, 2009), or chromatographic ones. Among the chromatographic techniques, special attention should be paid to the identification of specific minority components such as volatile compounds (Kádár et al., 2011; Juan-Borrás et al., 2014).

While some physicochemical parameters (colour, moisture, acidity, etc.) can vary between types of honey, contributing to some extent to their organoleptic characteristics, it is evident that in monofloral honey, flavour/aroma is the most distinctive pattern. Although all types of honey have a common intrinsic flavour/aroma, every nectar from blossom or secretions from plants give different specific aromas and flavours that strongly influence their distinguishing features. It has been shown that different honeys have certain specific compounds which some authors consider to be "fingerprints" or "markers", which may be useful in their botanical classification (Kádár et al., 2011; Juan-Borrás et al., 2014; Alissandrakis, Tarantilis, Harizanis, & Polissio, 2007; Kaskoniene & Venskutonis, 2010; Castro-Vázquez, Leon-Ruiz, Alañon, Pérez-Coello, & González-

Porto, 2014). For instance, methylanthranilate is important for the identification of orange blossom honey since this compound is only present in orange blossom nectar (Juan-Borrás, Domenech, & Escriche, 2015); likewise, formaldehyde and acetaldehyde in rape and clover honey, respectively; acetone in fir honey and diketones, sulphur compounds and alkanes in eucalyptus honey, among others (Bouseta, Collins, & Dufour, 1992; Da Silva, Gauche, Gonzaga, Costa, & Fett, 2016).

Currently, the beekeeping sector is aware of the importance of increasing the offer of monofloral honey, especially from a particular geographical origin. For this reason, whenever it is possible, honey is marketed specifying its botanical origin on the label in order to inform consumers and to increase producers' profit margins. Guarantying authenticity and differentiated quality, means that companies can increase the range of honey varieties on the market.

Although there are numerous works related to pollen, physicochemical parameters, and the volatile profile of different types of unifloral honey; as far as the authors know, there is no research focused on the relationship between this information and what appears on the label in terms of monoflorality. Therefore, the objective of this study was to investigate the correlation between the proclaimed type of monofloral honey (lavender or thyme) as appears on the label with the actual percentage of pollen, physicochemical parameters, colour, olfato-gustatory profile, as well as, volatile compounds. All this with the aim of evaluating the effectiveness of these techniques in the accurate classification of these monofloral honeys.

2. Material and methods

2.1.Honey samples

Honey samples, labelled as lavender or thyme and harvested in 2015 from the Valencian Region (Spain), were purchased locally from different retail outlets. Three batches of each

available brand on the market (11 lavender and 10 thyme) were acquired. Therefore, a total of 66 honey samples were analyzed.

2.2.Melissopalynological analysis

The percentage of pollen from lavender and thyme present in each sample was quantified following the criteria of the International Commission for Bee Botany (Von Der Ohe, Persano-Oddo, Piana, Morlot, & Martin, 2004). A light microscope (Zeiss Axio Imager, Göttingen, Germany) at a magnification power of ×400 with DpxView LE image analysis software attached to a DeltaPix digital camera was used. Figure 1 shows micrographs of grains of pollen of *Lavandula* spp. (A) and *Thymus* spp. (B) at 400× magnification. Light micrographs are shown in the top row (A1and B1) and DIC (differential interference contrast optics) micrographs at the bottom (A2 and B2).

2.3.Physicochemical and colour analysis

Hydroxymethylfurfural content (HMF), moisture content, conductivity, ºBrix and pH were analyzed in accordance with the Harmonized Methods of the European Honey Commission (Bogdanov, 2009). HMF was determined by HPLC-UV methodology using a ZORBAX Eclipse Plus C18 column (4.6 x 150mm, 5 μm particle size, Agilent Technologies, USA). Water-methanol (90:10, v:v), with a flow rate of 1 mL/min was used as a mobile phase. The detector was set to 285 nm. EZChrom Elite system software was used for HPLC data processing.

Water activity (a_w) was evaluated at 25 °C (± 0.2 °C) using an electronic dewpoint water activity meter, Aqualab Series 4 model TE (Decagon Devices, Pullman,Washington, USA), equipped with a temperature-control system.

Colour was measured using a millimetre Pfund scale (C 221 Honey Color Analyzer, Hanna Instruments).

122 *2.4.Volatile compound analysis: Extraction and GC–MS analysis*

123 Volatile compounds were extracted by purge and trap at 45 °C for 20 min and trapped in
124 a glass tube packed with Tenax TA (20–35 mesh), then purified nitrogen (100 mL min^{-1})
125 was bubbled through the sample (Escriche, Kadar, Juan-Borrás, & Domenech, 2011).
126 Next, the compounds were thermally desorbed at 220 °C for 10 min (at 10 mL min^{-1}
127 helium flow) (TurboMatrix TD, Perkin ElmerTM, CT-USA), cryofocused in a cold trap
128 at −30 °C, then the cold trap was heated to 250 °C (at a rate of 99 °C/s) which transferred
129 them onto the capillary column.

130 A GC–MS (Finnigan TRACETM MS, TermoQuest, Austin, USA) with a DB-WAX
131 capillary column (SGE, Australia) (60 m length, 0.32 mm i.d.,1.0 μm film thickness) was
132 used to separate the volatile compounds. Helium at a flow rate of 1 mL min^{-1} was used
133 as the carrier gas. The temperature programme was: from 40 °C (2-minute hold time) to
134 190 °C at 4 °Cmin^{-1} (11-minute hold time) and finally to 220 °C at 8 °C min^{-1} (8-minute
135 hold time). Electron impact mass spectra were logged in impact ionization mode at 70 eV
136 (mass range of m/z 33–433). A total of 3 extracts were obtained for each sample. 2-
137 Pentanol was used as an internal standard. The identification of isolated volatile
138 compounds was performed by comparing their mass spectra, retention times and linear
139 retention indices with those obtained from authentic standards from: Sigma-Aldrich (San
140 Louis, Missouri and Acros Organics, Geel, Belgium) and Fluka (Buchs, Schwiez,
141 Switzerland). The compounds for which it was not possible to find authentic standards
142 were tentatively identified by comparing their mass spectra (m/z values of the most
143 important ions) with spectral data from the National Institute of Standards and
144 Technology 2002 library (always considering more than 80% percent probability value),
145 as well as linear retention indices and spectral data published in the literature.

All the physicochemical, colour and volatile compound analysis were performed in triplicate.

2.5.Sensory analysis

The samples were evaluated organoleptically based on their monofloral olfato-gustatory profile. This analysis was carried out by experienced staff in the honey quality control laboratory at the Universitat Politècnica of València (Spain). A scale from 0 to 3 was used to score the perceived intensity (ISO, 2003). Tasters placed a small amount of honey (aprox 5 g) on their tongue, diluted it with saliva and projected it toward the back of their mouth to evaluate the flavour and aromas via the retronasal route. Then, the honey was swallowed slowly, and the taste persistence was evaluated. This procedure was followed for all samples.

2.6.Statistical analysis

An analysis of variance (ANOVA) (using Statgraphics Centurion for Windows) was applied to evaluate the physicochemical parameters, colour, volatile compounds, and phenolic and flavonoid compounds, according to the type of honey (lavender or thyme). The method used for multiple comparisons was the LSD test (least significant difference) with a significance level α=0.05. In addition, data were analyzed using a principal component analysis (PCA) applying the software Unscrambler X.10. The number of components extracted was based on the Kaiser criterion (1960) that suggests keeping all principal components with eigenvalues higher than 1 (Kaiser, 1960). This solution was not rotated using orthogonal or obliquus rotation. Subsequently, Stepwise Linear Discriminant Analysis (SLDA) was applied (using SPSS.18) to the loadings of these seven principal components with the aim of differentiating between varieties of honey.

169 This analysis permitted identification of the principal components with better predictive
170 power, by means of a unique discriminant function.

171 **3. Results and discussion**

172 *3.1. Physicochemical and colour characterization*

173 Table 1 shows the average values of the quantified physicochemical parameters (HMF,
174 moisture, electrical conductivity, ^{o}Brix, pH and a_w) and the colour of the three batches of
175 each brand commercially labelled as lavender or thyme honey.

176 The code for each brand refers to the average percentage of *Lavender* spp. (L) and
177 *Thyme* spp. (T) pollen. The organoleptic scores of these samples were based on the
178 monofloral (lavender or thyme) olfato-gustatory profile of the first named type of pollen,
179 the perception intensity being measured from absence (0) to very intense (3). For
180 example, the code "L1-T8 (2)" means that this brand has an average of 1% lavender
181 pollen and 8% thyme pollen, and the (2) represents an intermediate olfato-gustatory
182 intensity of lavender.

183 In the present work all the honey samples labelled as thyme satisfied the commercial
184 criterion about the percentage of pollen (minimum 10% of pollen *Thymus* spp.), since
185 they ranged from 11 to 16%. However, this was not the case for the group of samples
186 labelled as lavender, as the pollen ranged between 0 and 7% when their commercial
187 minimum criterion is also 10% *Lavandula* spp. pollen. The International Honey
188 Commission in a study carried out with 84 European lavender and 253 thyme honey
189 samples (Persano-Oddo & Piro, 2004), reported a slightly higher average percentage of
190 *Lavandula* spp pollen (between 1% and 19%) than those observed in the present work.
191 On the contrary, the average *Thymus* spp. pollen content was higher in Italian and Greek
192 thyme honey (26%±18 and 40%±16.4, respectively). The different species of thyme

involved in each case may be the main reason for these differences (Persano-Oddo & Piro, 2004). It seems evident that the "samples labelled as lavender" used in the present study are sold only following the criterion of aroma/flavour reminiscent of this flower (organoleptic analysis) since this type of sample did not met the pollinic criteria. This is not surprising since, as noted above, currently lavender honey is underrepresented in pollen due to the proliferation of hybrid varieties used in the perfume industry (Guyot-Declerck, Renson, Bouseta, & Collin, 2002; Von Der Ohe et al., 2004; ANIPAM, 2008; The Apis Information Resource Center website, 2016). Therefore, it is obvious that the pollen percentage criterion is unrealistic at least referring to lavender honey

This table also illustrates the ANOVA results (F-ratio and significant differences) obtained for the factor "type of labelled honey" carried out for the physicochemical parameters. Significant differences were not found between the two types of labelled honey (lavender and thyme) for any of the parameters analysed. This is because in both groups of samples all the parameters analysed are in the same range of values: moisture (13.73-19.60 and 15.60-17.60 g/100g); electrical conductivity (0.180-0.600 and 0.160-0.640 S/cm), ^{o}Brix (78.73-84.00 and 80.60-82.50); pH (3.78-4.40 and 3.83-4.80); a_w (0.49-0.60 and 0.48-0.58) and colour (40.00-89.00 and 47.00-84.00), respectively. These values agree with the results obtained for European lavender and thyme honey in the before mentioned study (Persano-Oddo & Piro, 2004), where the physicochemical reported data were: moisture (15.20-18.10 and 14.00-17.00 g/100g); electrical conductivity (0.120-0.310 and 0.250-0.540 S/cm); pH (3.50-4.00 and 3.50-4.10); and colour (20.30-45.00 and 35.00-74.5), respectively. It is important to point out that in the present work the colour Pfund for lavender honey was about double that in the European study, probably due to the influence of the vegetation present in the surroundings.

In relation to HMF a large amount of variation was observed: from 4.51 to 48.19 mg/kg in the case of lavender honey, and from 0.93 to 58.88 mg/kg for thyme honey. In both types of honey, two brands clearly exceeded the maximum of 40 mg/kg (Council Directive 2001/110 relating to honey, 2002), with values of 44.74 and 48.19 mg/kg in lavender honey, and 48.75 and 58.88 in thyme honey. This shows that these samples were not properly handled, or the time between harvesting and retail sale was too long.

The information given by the physicochemical parameters and the colour shows that neither of them permits differentiation between honey from the same geographical habitat labelled as lavender or thyme, as is the case of the two types of honey studied here.

The volatile compounds that are liberated during the tasting and ingestion of honey decisively influences the aroma/flavour perceived. Therefore, it is logical to think that the volatile fraction of honey contains potentially usable information for the differentiation of lavender honey from other types of honey with which it might be confused.

3.2. Olfato-gustatory profile and volatile compound characterization

Around 30 major volatile compounds were identified and semiquantified in the volatile fractions of honey samples, including alcohols, aldehydes, ketones, acids, esters, terpenes and nitrogen compounds. The average values, standard deviation, and the ANOVA result of the volatile compounds analysed in both types of honey are shown in Table 2.

Alcohols, as in other types of honey, were abundant in the analysed samples. All of them had 2-propanol, 2-butanol and 1-butanol in similar amounts. Ethanol was more common in thyme honey samples, although without significant differences between both groups. The alcohol 1-hexanol was present in significantly greater amounts in the samples labelled as lavender; some authors propose 1-hexanol, among other compounds, as a typical marker of lavender honey. In fact, in the present study 1-hexanol was identified

241 in almost all the brands labelled as lavender (average value of 4.2 μg/kg) and only in one
242 brand labelled as thyme (T16-L3) with an average value in this brand of 1.4 μg/kg. The
243 existence of lavender pollen in this last sample shows that the honeybees visited lavender
244 flowers too, which could explain the occurrence of 1-hexanol in this sample. The large
245 amount of 1-hexanol found in lavender honey is in line with the results obtained in other
246 studies of Spanish lavender honey (Castro-Vázquez et al., 2009; Castro-Vázquez et al.,
247 2014).

248 In the present study, the levels of methyl alcohols in the two groups of honeys were
249 not found to be significantly different by the ANOVA. However, among them, 2-methyl-
250 3-buten-2-ol, 3-methyl-3-buten-1-ol, and 2-methyl-2-buten-1-ol, where more abundant in
251 samples labelled as lavender and 2-methyl-1-propanol and 2-methyl-1-butanol in thyme
252 samples. Apparently, the methyl alcohols contribute to the typically fresh aroma of this
253 type of honey (Castro-Vázquez et al., 2009; Bouseta, Collins, & Dufour, 1992; Da Silva
254 et al., 2016).

255 Aldehydes such as 3-methyl-1-butanal, 2-methyl-1-butanal, hexanal, heptanal furfural
256 and phenylacetaldehyde, were also identified. Phenylacetaldehyde, described as having a
257 honey-like aroma was present in most of the thyme honey samples and in very few of
258 those labelled as lavender. This agrees with the results obtained for Greek thyme honey
259 (Alissandrakis et al., 2007; Karabagias, Badeka, Kontakos, Karabournioti, &
260 Kontominas, 2014), and for Spanish thyme honey (Castro-Vázquez et al., 2009).
261 However, other authors reported the importance of phenylacetaldehyde to characterize
262 lavender honey from different botanical species (Guyot-Declerck et al., 2002). In the
263 present study, only a few samples of lavender showed the presence of this compound.

Many authors considered that hexanal is one of the compounds most responsible for the characteristic flavour of lavender honey (Bouseta, Collins, & Dufour, 1992; Guyot-Declerck et al., 2002; Manyi-Loh, Roland, & Clarke, 2011). In fact, in the present work the importance of hexanal is of note as it appeared in almost all the samples labelled as lavender (reaching values of 5.0 μg/kg) and in T16-L3. Although this sample had a high enough percentage of pollen to be classified as thyme, it is noteworthy that it had the organoleptic characteristics and aromatic notes typical of lavender honey, as well as the presence of pollen from this plant.

The two groups of analysed samples showed opposing behaviour in terms of methyl aldehydes with significant differences between them; 3-methyl-1-butanal was almost exclusively found in the group of honey samples labelled as lavender, whereas 2-methyl-1-butanal in those of thyme.

Different ketones were present in almost all the samples analysed: acetone; 2-butanone, 2,3 butanedione 1-hydroxy-2-propanone, and 3-hydroxy-2-butanone. In general, ketones are very common in different types of European honey, acetone being one of the major volatile compounds detected (Da Silva et al., 2016). In the present work, all the ketones identified were more abundant in thyme honey than honey labelled as lavender, although only for acetone the differences were significant between the two groups.

Acetic acid and ethyl acetate were the only acid and ester identified, respectively. Acetic acid showed significant differences between groups, being present in all the samples labelled as lavender (average value of 2.3 μg/kg) and only in three of the thyme samples. However, not significant differences were found for ethyl acetate.

β-linalool and hotrienol (3,7-dimethyl-1,5,7 octatrien-3-ol) were the only honey terpenes identified in the present work. Different studies reported that the derivatives of β–linalool originated from flowers visited by honeybees are found only in specific types of honey (Da Silva et al., 2016). Several authors highlighted the importance of hotrienol in lavender honey, compared to other types of honey (Castro-Vázquez et al., 2009; Castro-Vázquez et al., 2014; Jerkovic & Kus, 2014). In the present work, β–linalool was identified in both honey samples, although was significantly higher in those of thyme, whereas hotrienol was almost exclusively found in the samples labelled as lavender (reaching values up to 4.8 μg/kg). Other volatile terpenes such as thymol or carvacrol, which were reported by other authors in thyme honey, were not found in this study. This was probably due to the different botanical species or the analytical extraction procedures applied for these compounds (Cacho, Campillo, Viñas, & Hernández-Córdoba, 2015).

Only four brands labelled as thyme showed small amounts of short-chain nitrogen compounds (2-methyl-propanenitrile, 2-methyl-butanenitrile). Unsurprisingly, nitrile derivatives could be present in honey samples, even becoming important compounds in the headspace fraction (Moreira & De Maria, 2005), in some cases reaching 21.7% of the total volatile compounds (Kaškoniene, Venskutonis, & Ceksteryte, 2008). For instance, phenylacetonitrile was reported as very abundant in thyme honey from Greece (Alissandrakis et al., 2007).

With the aim of transforming the initial set of volatile compound variables into a more reduced set of linearly uncorrelated variables, a principal component analysis was performed. This analysis was carried out using the average values from the three repetitions for each sample of honey. Seven components were extracted according to the Kaiser criterion (1960) (Kaiser, 1960), explaining 95.5% of the total variance. The first

component (PC1) explained 40% and was positively correlated with ethyl acetate, 2,3-butanedione, 2-methyl-propanenitrile, 2-methyl-1-propanol, 3-methyl-butanenitrile, 1-butanol, 2-methyl-1-butanol, among others. The second component (PC2 explained 22.0%) was positively correlated with 1-hexanol, hotrienol, hexanal, acetic acid and 2-methyl-2-buten-1-ol, and others. Figure 2 shows the scores and loadings for the two principal components. The score codes correspond to those explained in Table 1. Proximity between samples labelled as lavender or thyme, indicates similar behavior in terms of the volatile profile. The loading plot confirms that certain compounds are responsible for differentiation between the two groups. Ethyl acetate, 2,3-butanedione, 2-methyl-propanenitrile and 1-butanol, associated with PC1, as well as 1-hexanol, hotrienol, hexanal, corresponding to PC2, are characteristic of thyme and lavender honey, respectively.

Furthermore, the third component (11.5%) was positively correlated to octane and 3-methyl-3-buten-1-ol, while the fourth (8.6%), fifth (5.8%), sixth (4.4%) and seventh (3.2%) components were mainly correlated with ethanol, furfural, acetone and 2-methyl-butanal, and 3-methyl-butanal, respectively.

Once the variability of the initial set of volatile compound variables was reduced to seven principal components, a discriminant analysis was applied to examine the predictive power of each principal component when distinguishing between groups. That is to say, the previous seven principal components extracted using PCA were subsequently used as predictors of honey type in the discriminant analysis. As a result of this analysis, only one statistically significant canonical function was obtained. This function explained 100% of the total variance (Wilks' lambda=0.462, df=7, p=0.101; Canonical correlation coefficient=0.734), and also discriminates correctly between honey

335 labelled as lavender or thyme in 85.7% of the samples. The discriminant function values
336 at the group centroids were -1.186 for lavender and 0.890 for thyme.

337 The standardized coefficients of discriminant functions for each principal component
338 used as a predictor were: PC1 (0.731); PC2 (-0.819); PC3 (0.323); PC4 (0.227); PC5 (-
339 0.586); PC6 (0.363) and PC7 (-0.088). These data reveal that the second component
340 showed the highest predictive power of the discriminant function, followed by the first
341 component. This shows that the compounds most involved in the distinction between the
342 two types of honey were: 1-hexanol, hotrienol, hexanal, acetic acid and 2-methyl-2-
343 buten-1-ol.

344 The results of the discriminant analysis are shown in Figure 3. Next to the code for
345 each sample appears the organoleptic score based on its monofloral (lavender or thyme)
346 olfato-gustatory profile as was described in Table 1. Samples of thyme honey fell in the
347 left region of the map, whereas the rest of the samples were placed on the right, though
348 both varieties obtained negative scores for this discriminant function.

349 Table 3 summarizes the information concerning commercial labelling and possible
350 classifications of samples according to several criteria: percentage of pollen, olfato-
351 gustatory profile and volatile compound obtained from discriminant analysis.
352 Additionally, chi–square tests are shown to assess the relationship between commercial
353 labelling and the classification provided by each criterion.

354 None of the samples labelled as lavender should have been classified as such according
355 to the pollen content (minimum 10% of *Lavandula* spp. pollen). Actually, they should be
356 classified as polyfloral with the exception of two of them that could be classified as thyme
357 honey. However, the organoleptic perception (in 81.8% of these samples) and the volatile
358 profile (in 90.9%) conform to the information given on the samples labelled as lavender.

Regarding thyme honey, the information on the label is correct for all samples considering the pollen content (100%). With the exception of one sample, all of them showed the characteristic olfato-gustatory profile and volatile profile of thyme honey (90.0% in both cases). Chi–square tests confirm that there is a significant association between the commercial labelling and the classification given by the organoleptic perception ($\chi 2$=10.83, p=0.001) and overall by the volatile compounds ($\chi 2$=13.74, p=0.000).

4. Conclusion

This paper highlights the importance of a detailed review of the information that appears on commercial labels. Besides, the need to identify alternative analytic techniques to help organizations provide more accurate content in their labelling processes is underlined.

This work contributes to reinforcing the usefulness of the volatile fraction of honey to provide more accurate honey labelling. The results offer compelling evidence that the volatile compound profile can be used for the classification of lavender type honey since this technique agrees quite well with the organoleptic analysis. The volatile analysis has a clear advantage over the pollinic one in the case of lavender honey.

In general, when pollen content is under-represented, volatile analysis could be a complementary technique to the pollinic one. Obviously, this analysis would only be recommended when honey has specific identifiable organoleptic characteristics.

The present findings have important implications for solving problems in the honey sector regarding the correct classification of underrepresented monofloral honey. Organizations may benefit from this new approach to volatile information, and consumers may buy honey products with guaranteed botanical origin. Considering these results, there are still some undefined sources of unreliability that may influence the final classification of samples containing a mixture of lavender and thyme characteristics. We

383 are aware that the research needs to be expanded to include a greater number of samples
384 from a wider time period.

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Pollen viability and natural hybridization of Central European species

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Figure captions

Figure 1. Pictures at 400× magnification of pollen of Lavandula spp. (A) and Thymus spp. (B). A1 and B1- Light micrographs. A2 and B2 - DIC (differential interference contrast optics) micrographs.

Figure 2. (2.a) Scores (brand samples) and (2.b) loading (volatile compounds) plots of the first two components. (L) and (T) = percentage of *Lavender* spp. and *Thyme* spp. pollen.

Figure 3. Score plots of the first two components of the PCA-DA model. (L) and (T) = percentage of Lavender spp. and Thyme spp. pollen, (0-3)=intensity of organoleptic perception of the first named honey.

Table 1. Olfato-gustatory profile and average values of the physicochemical parameters and colour of each of the brands commercially labelled as lavender or thyme honey. L= lavender; T= thyme. Brand codes refer to the average percentage of pollen of *Lavandula* spp. (L) and (*Thymus* spp.). The organoleptic score of the brands was based on their monofloral (lavender or thyme) olfato-gustatory profile, perception intensity from 0 (absence) to 3 (very intense).

Brand Codes	Olfato-gustatory profile	Commercial information	Moisture (g/100g)	Electrical conductivity S/cm	ºBrix	pH	a_w	Colour	HMF (mg/kg^{-1})
L1-T8	(2)lavander	lavander	15.60 (0.01)	0.250 (0.006)	82.65 (0.01)	3.95 (0.01)	0.53 (0.01)	47 (1)	4.51 (0.01)
L3-T12	(3)lavander	lavander	15.27 (0.12)	0.330 (0.001)	83.07 (0.06)	3.94 (0.01)	0.49 (0.01)	66 (1)	15.49 (1.9)
L3-T0	(1)lavander	lavander	18.07 (0.12)	0.330 (0.001)	80.40 (0.01)	3.85 (0.01)	0.60 (0.01)	89 (1)	44.74 (1.6)
L7-T0	(3)lavander	lavander	14.80 (0.01)	0.180 (0.001)	83.40 (0.01)	3.78 (0.01)	0.54 (0.01)	80 (1)	48.19 (1.6)
L1-T26	(0)lavander; (1)thyme	lavander	15.40 (0.01)	0.600 (0.001)	82.82 (0.08)	4.40 (0.01)	0.53 (0.01)	81 (1)	10.09 (0.8)
L3-T0	(1)lavander	lavander	15.60 (0.01)	0.500 (0.015)	82.62 (0.03)	4.23 (0.01)	0.52 (0.01)	77 (1)	8.48 (0.2)
L4-T0	(2)lavander	lavander	16.60 (0.01)	0.320 (0.010)	81.53 (0.06)	4.02 (0.01)	0.55 (0.01)	61 (1)	4.85 (0.2)
L5-T0	(3)lavander	lavander	19.60 (0.01)	0.480 (0.010)	78.73 (0.06)	4.05 (0.01)	0.59 (0.01)	51 (1)	4.75 (0.5)
L1-T8	(0)lavander; (1)thyme	lavander	15.80 (0.01)	0.490 (0.006)	82.43 (0.03)	3.98 (0.01)	0.50 (0.01)	70 (1)	8.81 (0.5)
L0-T0	(1)lavander	lavander	17.40 (0.01)	0.250 (0.010)	80.80 (0.01)	3.89 (0.01)	0.55 (0.01)	40 (1)	8.40 (1.3)
L0-T0	(1)lavander	lavander	13.73 (0.12)	0.350 (0.006)	84.00 (0.01)	4.23 (0.01)	0.49 (0.01)	68 (1)	16.48 (0.9)
T16-L3	(1)thyme; (1)lavander	thyme	16.60 (0.01)	0.330 (0.006)	81.60 (0.01)	3.84 (0.01)	0.58 (0.01)	60 (1)	4.29 (0.4)
T14-L0	(1)thyme	thyme	17.60 (0.01)	0.570 (0.006)	80.60 (0.01)	4.11 (0.01)	0.58 (0.01)	74 (1)	0.93 (0.3)
T14-L0	(2)thyme	thyme	15.60 (0.01)	0.400 (0.015)	82.50 (0.01)	4.80 (0.01)	0.55 (0.01)	78 (1)	1.63 (0.5)
T12-L0	(1)thyme	thyme	16.80 (0.01)	0.420 (0.017)	81.60 (0.01)	3.94 (0.01)	0.56 (0.01)	71 (1)	6.07 (0.7)
T11-L0	(1)thyme	thyme	15.60 (0.01)	0.420 (0.021)	82.50 (0.01)	4.18 (0.01)	0.49 (0.01)	78 (1)	10.18 (1.2)

T12-L0	(1)thyme	thyme	16.60 (0.01)	0.160 (0.001)	81.60 (0.01)	3.83 (0.01)	0.49 (0.01)	47 (1)	58.88 (0.9)
T11-L0	(1)thyme	thyme	17.60 (0.35)	0.340 (0.006)	80.67 (0.09)	3.85 (0.01)	0.53 (0.01)	84 (1)	48.75 (1.3)
T11-L0	(1)thyme	thyme	15.80 (0.20)	0.500 (0.001)	82.40 (0.08)	3.90 (0.01)	0.48 (0.01)	72 (1)	8.52 (0.6)
T13-L0	(1)thyme	thyme	16.20 (0.01)	0.340 (0.006)	82.03 (0.06)	3.91 (0.01)	0.56 (0.01)	81 (1)	12.44 (0.3)
T13-L0	(1)thyme	thyme	16.40 (0.01)	0.640 (0.006)	81.70 (0.01)	4.31 (0.01)	0.58 (0.01)	69 (1)	6.64 (0.7)
ANOVA									
F-ratio			2.73ns	1.46ns	2.16ns	0.26ns	0.15ns		

ns: Non significant

Table 2. Volatile compounds (average values and standard deviation) in samples labelled as lavender or thyme honey. The data were calculated (μg/kg of honey) assuming a response factor equal to one.

VOLATILE COMPOUNDS	RI cal	ID	Samples labelled as lavender Mean (SD)	Samples labelled as thyme Mean (SD)	ANOVA F ratio
Alcohols					
2 Propanol	947	MS;RI	1.2 (1.1)	1.6 (1.5)	0.3^{ns}
Ethanol	957	MS;RI	20.3 (13.3)	38.1 (20.6)	3.6^{ns}
2 Butanol	1047	St;MS;RI	8.5 (6.1)	6.4 (9.0)	0.2^{ns}
2 Methyl-3-buten-2 ol	1065	MS;RI	6.1 (3.3)	5.2 (1.9)	0.5^{ns}
1 Butanol	1175	St;MS;RI	2.4 (3.1)	3.8 (6.5)	0.2^{ns}
2 Methyl-1-propanol	1119	St;MS;RI	6.7 (2.3)	9.5 (2.3)	0.69^{ns}
2 Methyl-1-butanol	1185	St;MS;RI	7.7 (4.4)	8.9 (5.9)	0.2^{ns}
3 Methyl-3-buten-1-ol	1277	St;MS;RI	6.3 (2.9)	5.2 (2.4)	0.78^{ns}
2 Methyl-2-buten-1-ol	1349	MS;RI	3.0 (2.1)	1.4 (1.3)	3.2^{ns}
1 Hexanol	1476	St;MS;RI	4.2 (2.2)	0.1 (0.5)	15.9^{**}
Aldehydes					
3-methyl-1-butanal	912	St;MS;RI	1.6 (2.4)	<0.001	2.37^{*}
2-methyl-1-butanal	920	MS;RI	0.3 (0.5)	2.6 (2.5)	6.9^{*}
Hexanal	1065	St;MS;RI	3.2 (2.4)	0.6 (1.2)	8.9^{**}
Heptanal	1160	St;MS;RI	9.2 (6.9)	19.7 (2.8)	1.35^{ns}
Furfural	1460	St;MS;RI	3.2 (4.6)	2.9 (4.5)	0.02^{ns}
Phenylacetaldehyde	1609	MS;RI	1.0 (1.0)	2.8 (1.6)	1.8^{ns}
Ketones					
Acetone	814	MS;RI	1.0 (0.3)	2.0 (0.9)	4.7^{*}

2-Butanone	910	St;MS;RI	1.7 (0.7)	2.6 (1.9)	2.2^{ns}
2,3 Butanedione	970	MS;RI	2.8 (1.9)	7.4 (7.7)	3.5^{ns}
1-Hydroxy-2-propanone	1268	MS;RI	2.2 (1.2)	2.9 (1.4)	1.2^{ns}
3-Hydroxy-2-butanone	1322	St;MS;RI	2.6 (1.4)	4.8 (3.0)	1.9^{ns}
Acids					
Acetic acid	1486	St;MS;RI	2.3 (1.4)	0.9 (0.5)	5.4*
Esters					
Ethyl acetate	909	St;MS;RI	1.1 (1.4)	1.4 (1.2)	0.6^{ns}
Terpenes					
β-Linalool	1670	St;MS;RI	3.6 (0.15)	4.9 (0.15)	4.8*
Hotrienol	1737	MS;RI	2.7 (1.1)	0.3 (0.2)	35.8***
Nitrogen compounds					
2-Methyl-propanenitrile	1022	St;MS;RI	<0.001	0.23 (0.35)	2.5^{ns}
2-Methyl-butanenitrile	1158	MS;RI	<0.001	0.17 (0.27)	3.9^{ns}

ns: Non significant; *P<0.05; **P<0.01; ***P<0.001

RI cal: Linear retention indices calculated

ID: Method of identification, MS (comparison with mass spectrum stored in NIST library), St (comparison of retention time and mass spectrum with those of authentic standards), RI (comparison of linear retention indices with the literature)

Table 3. Comparison of commercial labelling and possible classifications of samples according to presence of pollen, organoleptic profile and volatile compounds.

Brand Code	Commercial labelling	Pollen	Olfato-gustatory profile	Volatile compound
L1-T8	Lavender	Polyfloral	Lavender	Lavender
L3-T12	Lavender	Thyme	Lavender	Lavender
L1-T8	Lavander	Polyfloral	Thyme	Thyme
L3-T0	Lavander	Polyfloral	Lavander	Lavander
L7-T0	Lavander	Polyfloral	Lavander	Lavander
L1-T26	Lavander	Thyme	Thyme	Lavander
L3-T0	Lavander	Polyfloral	Lavander	Lavander
L4-T0	Lavander	Polyfloral	Lavander	Lavander
L5-T0	Lavander	Polyfloral	Lavander	Lavander
L0-T0	Lavander	Polyfloral	Lavander	Lavander
L0-T0	Lavander	Polyfloral	Lavander	Lavander
T14-L0	Thyme	Thyme	Thyme	Thyme
T14-L0	Thyme	Thyme	Thyme	Thyme
T12-L0	Thyme	Thyme	Thyme	Thyme
T11-L0	Thyme	Thyme	Thyme	Thyme
T12-L0	Thyme	Thyme	Thyme	Thyme
T11-L0	Thyme	Thyme	Thyme	Thyme
T11-L0	Thyme	Thyme	Thyme	Thyme
T13-L0	Thyme	Thyme	Thyme	Thyme
T16-L3	Thyme	Thyme	Lavander	Lavander
T13-L0	Thyme	Thyme	Thyme	Thyme
% of samples conform the information given on the Lavander label	-	0%	81.8%	90.9%
% of samples conform the information given on the thyme label	-	100%	90.0%	90.0%

χ^2 (p-value)	-	-	10.83 (0.001)	13.74 (0.000)

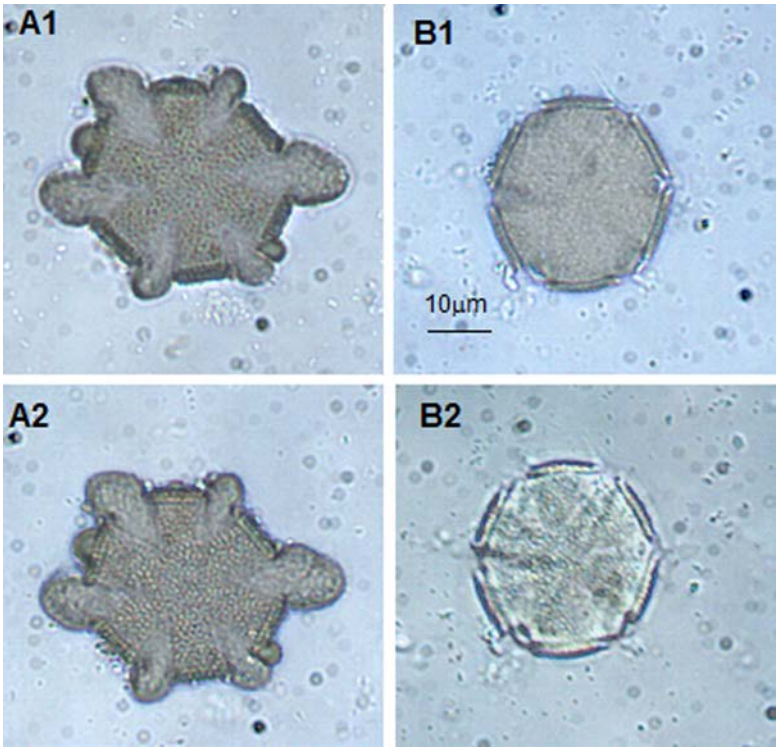


Figure 1

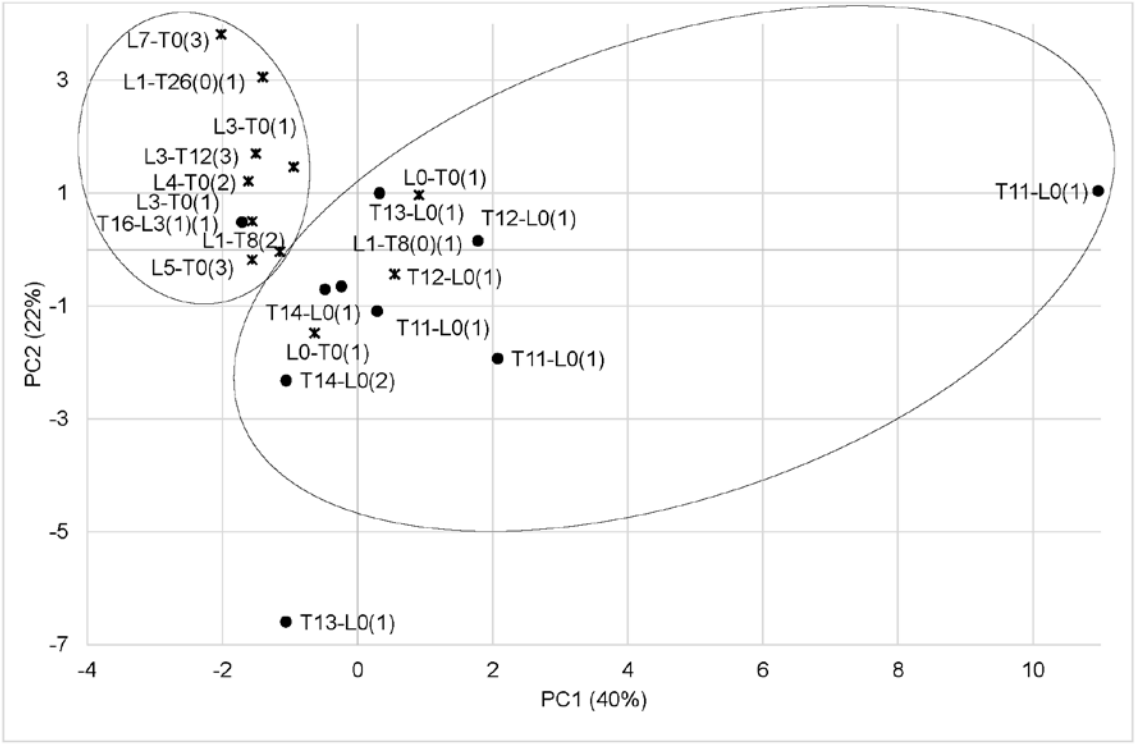
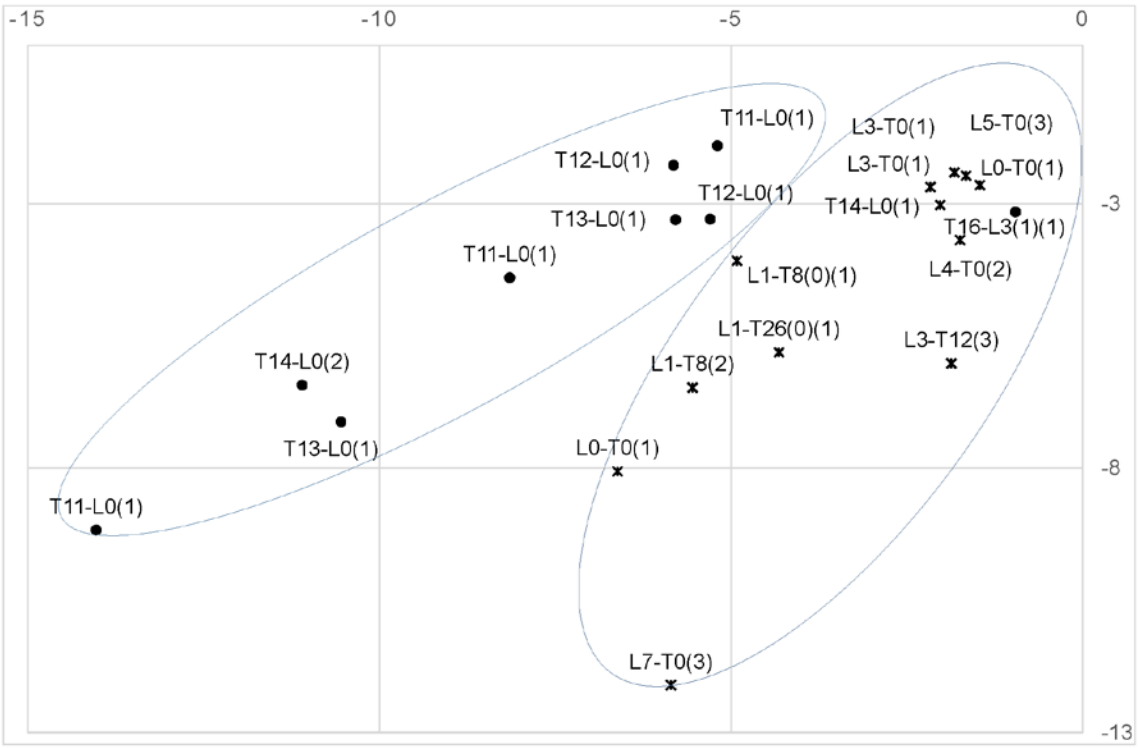

L7-T0(3)
L1-T26(0)(1)
L3-T0(1)
L3-T12(3)
L4-T0(2)
L3-T0(1)
T16-L3(1)(1)
L1-T8(2)
L5-T0(3)
L0-T0(1)
T13-L0(1)
T12-L0(1)
L1-T8(0)(1)
T12-L0(1)
T14-L0(1)
T11-L0(1)
L0-T0(1)
T11-L0(1)
T14-L0(2)
T13-L0(1)
T11-L0(1)
PC2 (22%)
PC1 (40%)
3
1
-1
-3
-5
-7
-4
-2
0
2
4
6
8
10

Figure 2



19
20
21 Figure3