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# Description of the volatile fraction of *Erica* honey from the northwest of the Iberian Peninsula

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ABSTRACT

Heather honey is highly appreciated by consumers for its sensorial profile, which varies depending on the flora used by the honeybees. Volatile compounds contribute to these qualities. Characterisation of the volatile profile related to the botanical origin is of great interest for the standardization of unifloral honey. For this reason, 33 heather honey samples from northwest of the Iberian Peninsula were analysed by headspace solid-phase microextraction (HS-SPME) to identify the key volatile compounds in this type of honey. The aim of this research was to provide a descriptive analysis of these compounds, and to find whether there is any relationship with the main *Erica* species. A total of 58 volatile organic compounds were found, with hotrienol, phenylacetaldehyde, and *cis*-linalool being the most abundant. A principal component analysis and Spearman's rank correlation showed the homogeneity of the volatile profile in the samples, and their close relationship with the main pollen types.

## 1. Introduction

The production of honey and other apicultural products is an ancient activity with economic importance worldwide (Manyi-Loh, Ndip & Clarke, 2011). Honey, the most important primary food of beekeeping derived from nectar and/or honeydew, contains sugars as a major component. However, it has also some valuable nutrients, such as vitamins, minerals, enzymes, flavouring organic compounds, free amino acids and numerous volatile compounds, as minor components (Manyi-Loh et al., 2011; Escuredo, Míguez, Fernández-González & Seijo, 2013). The composition of honey and its sensorial properties are strongly associated with the plants visited by the honeybees and its geographical origin, since the climate and soil determine the distribution of the plants (Castro-Vázquez, Díaz-Maroto, De Torres & Pérez-Coello, 2010; Manyi-Loh et al., 2011).

The Northwest of the Iberian Peninsula is an important area of production of heather honey. Several different species of *Erica* plants contribute to the production of this honey type, especially *Erica umbellata*, *E. arborea,* and *E. cinerea,* among others. These plants are very abundant in mountainous areas and form monospecific scrublands or, together with other plants such as *Ulex*, *Cytisus* and *Genista*, mixed plant formations, which are very well represented in the area (Seijo & Jato, 1998; Rodríguez-Flores, Escuredo, Seijo-Rodríguez & Seijo, 2019). The honey obtained from these plants has sensorial properties that are highly appreciated by consumers. These include a dark amber to dark colour with reddish tones, persistent and slightly bitter taste, and vegetal smell reminiscent of wet soil or wet leaves in soil, sometimes with floral perceptions.

The volatile profile is one of the most important characteristics of a food product, since it has a notable influence on the organoleptic profile and supports its authenticity (Radovic, Careri, Mangia, Musci, Gerboles & Anklam, 2001). The volatile substances in honey form a complex mixture that depends on the source area, nectar, processing and storage conditions, and the action of honeybees and microorganisms (Castro-Vázquez, Díaz-Maroto, González-Viñas & Pérez-Coello, 2009; Escuredo, Dobre, Fernández-González & Seijo, 2014; da Silva, Gauche, Gonzaga, Costa & Fett, 2016). Although many volatile compounds can be found in the honey samples, some of them are specific, giving certain honeys a particular fingerprint. In fact, specific markers for honey types are being investigated. For this reason, the characterisation of the volatile compounds of honey is a topic of great interest in beekeeping, since it can contribute to the discrimination and characterisation of the product (Karabagias, Papastephanou & Karabagias, 2019).

According to previous studies, more than 600 different compounds

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have been identified in the volatile fraction of honey (Cuevas-Glory, Pino, Santiago & Sauri-Duch, 2007; Kaškonienė & Venskutonis, 2010; Manyi-Loh et al., 2011). However, the number of these identified compounds is likely to increase, since there are unifloral honeys that have not yet been characterized by their volatile composition, and due to the continuous improvement of the sensitivity of the techniques of analysis. Additionally, the extraction method plays an important role in the quantification and identification of volatile compounds. An association of both main techniques, GC (gas chromatography) and MS (mass spectrometry), results in a combined GC–MS technique that allows the separation and identification of complex mixtures. This technique is a powerful tool to separate, identify, and quantify volatile and semi-volatile components. However, the methods for the extraction of volatile components are prone to sample loss and degradation, and require many steps and a lot of time. Solid-phase microextraction (SPME) can eliminate these problems. This is a relatively new, rapid, solvent-free extraction technique that can be used with GC (Arthur & Pawliszyn, 1990). Its straightforward handling makes it perfect for working with a matrix as complex as the volatile substances of honey (Cuevas-Glory et al. 2007).

Normally, unifloral honey has more uniform volatile profiles compared to multifloral honey. Several studies have tried to characterize unifloral honey of various origins by determining their volatile profiles, in search of specific chemical marker compounds (Castro-Vázquez et al., 2009; de la Fuente, Martínez-Castro & Sanz, 2005; Guyot, Scheirman & Collin, 1999; Kaškonienė & Venskutonis, 2010; Manyi-Loh et al., 2011; Piasenzotto, Gracco & Conte, 2003). Previous studies on heather honey from *Erica arborea* described volatile compounds such as α-isophorone, 2-hydroxy-3,5,5-trimethylcyclohexanone, furfuryl alcohol, benzyl alcohol and 2-phenylethanol (de la Fuente et al., 2005; Kaškonienė & Venskutonis, 2010; Karabagias, Maia, Karabagias, Gatzias & Badeka, 2018).

Heather honey from the northwest of the Iberian Peninsula comes mainly from *Erica umbellata* and *E. arborea*, but honeybees can use other *Erica* species to produce honey. Due to the influence of volatile compounds on the sensory properties of honey, a detailed investigation of the volatile profile of heather honey could contribute to its characterisation. Thus, this work aims to study the compounds of the volatile fraction of heather honey from the northwest of the Iberian Peninsula, and their relationship with the surrounding flora.

## 2. Materials and methods

### 2.1. Honey samples

A total of 33 fresh heather honeys produced in the northwest of the Iberian Peninsula (Spain and Portugal) were provided directly by the beekeepers. Samples were obtained during the 2018 and 2019 harvests. The extractions were carried out on fresh samples. These were kept frozen during storage until the volatile extraction date. Melissopalynology was used to confirm the botanical origin of the samples.

### 2.2. Palynological analysis

The qualitative palynological study of the honey samples was performed according to the method used in Rodríguez-Flores, Escuredo, Seijo-Rodríguez, & Seijo, (2019). Ten grams of honey were dissolved in double distilled water and centrifuged at 4500 rpm for 10 min. The obtained sediment was re-dissolved and centrifuged for an additional 5 min. The final volume of the sediment was used to prepare a slide for the microscopic study. The different pollen types and their relative frequencies (pollen spectra of the honey sample) were determined using a Nikon Optiphot II microscope (Nikon UK Ltd., London, UK) at 400x or 1000x (when needed). The results were expressed as the percentage of the pollen type over the total pollen counted and identified in the sample.

### 2.3. Volatile extraction by solid phase microextraction (SPME)

A 7.5 g honey sample was introduced into a 20 mL vial, before adding 7.5 mL of a 30% sodium chloride solution. After placing a magnetic stirrer, the vial was sealed. Then, the sample was stirring until homogenisation was achieved and placed into a thermostatic bath at 50 °C. A 65 μm thick polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber (Supelco SPME fibre 57326U, Darmstadt, Germany) was used for the extraction and subsequent analysis of the volatile compounds by SPME. Before use, the SPME fiber was preconditioned and thermally cleaned. This was done thermally by exposing the fiber to a conditioning temperature of 250 °C for 50 min in the GC injection port. The chosen extraction mode was Headspace (HS), since the analytes of interest were highly volatile. Thus, the fiber was introduced into the vial and exposed to the headspace under the sample for 60 min. After this period, the fibre was retracted and transferred to the gas phase chromatograph (GC) injector, where the compounds were desorbed for 5 min.

### 2.4. Chromatographic analysis by GC–MS

The analysis of volatile compounds previously extracted by SPME was performed using a GC–MS Perkin Elmer System with a GC module Claurus® 580 GC and an MS Claurus® SQ 8 S module (PerkinElmer Inc., Massachusetts, USA). The injection was made in splitless mode, and the fibre desorption was carried out for 5 min at 250 °C. The compounds were separated on a DB-5MS column (30 m × 0.25 mm i.d., thickness 0.25 um; J & W Scientific, Inc.). The oven temperature was programmed from 40 °C to 170 °C (3 °C/min) and from 170 °C to 290 °C (25 °C/min), then was maintained at 290 °C for 15 min. Helium was used as carrier gas at a constant velocity of 40 $cm.s^{-1}$. The mass spectrum was obtained from an ionization energy of 70 eV. The transfer line and the ionization source temperatures were 250 °C and 230 °C, respectively. TurboMass Ver6.1.0 6.1 software (PerkinElmer Inc., Massachusetts, USA) was used to acquire the data. The assignment of the chromatographic peaks was performed using a commercial MS database (NIST 2011 mass spectral library). Linear retention indices (LRI) were calculated for each component detected. This allowed us to confirm the identification of each compound. For the calculation of the LRI indices, a mixture of *n*-alkanes ($C_7$–$C_{40}$) (Sulpelco, Bellefonte, PA, USA) dissolved in hexane was used. The values of the relative areas (percentage of total volatiles) were obtained directly from the total ion current chromatogram (Total Ion Chromatogram, TIC).

### 2.5. Statistical analysis

The data were analysed using the statistical programs IBM SPSS Statistics 23.0 (IBM, UK) and STATGRAPHICS Centurion XVIII (Statgraphics Technologies, Inc., Virginia, USA). Principal component analysis was carried out to show the homogeneity of the samples and relationships between the VOCs and the main pollen types. Furthermore, these relationships were corroborated by a Spearman's rank correlation. These correlation coefficients measure the strength of the association between the variables. The *P*-value shows the statistical significance of the estimated correlations.

## 3. Results and discussion

### 3.1. Pollen spectra of the honey samples and predominance of Erica species

The pollen profile of a honey sample commonly contains many pollen types from the flowering plants of the neighbourhood where honey was produced. Even if the sample is considered a unifloral honey, different pollen grains can appear, leading to a high biodiversity of

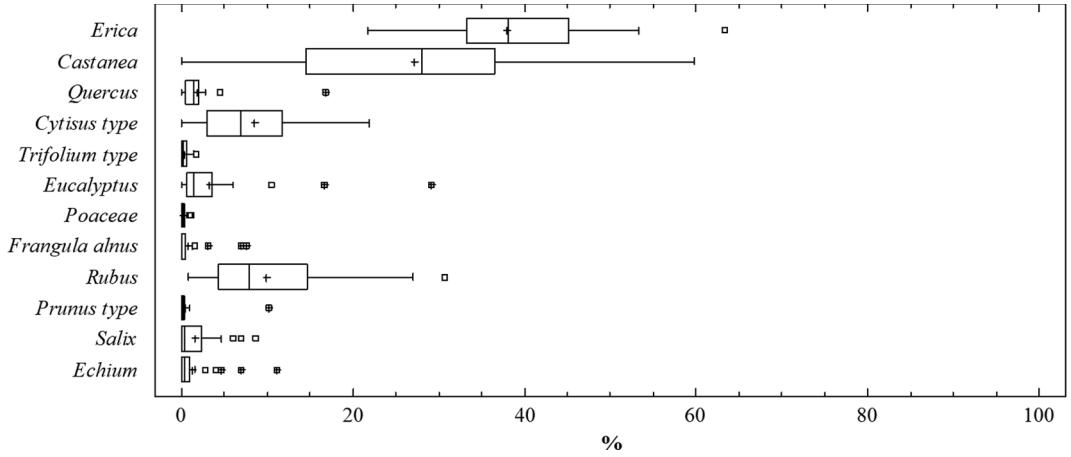


**Fig. 1.** Main pollen types found in heather honey samples with a percent of over 50%

pollen types. In the studied honeys, 79 pollen types were identified. The most representative was *Erica* pollen, which was always present at a high percentage. The pollen of *Castanea*, *Quercus, Cytisus* type, *Trifolium* type, *Eucalyptus*, Poaceae, *Frangula alnus*, *Rubus*, *Prunus* type, *Salix* and *Echium* were the most representative in this group of honeys, found in more than 50% of the samples (Fig. 1). The average value of *Erica* pollen was 37.9%, and the maximum value was 63.3%. *Erica* pollen can appear in unifloral honey at values of < 45% (the considered value in melissopalynology for dominant pollen), as occurs with other plants of the Ericaceae family (Persano-Oddo, Piro, Bruneau, Guyot-Declerck, Ivanov, Piskulová & Von der Ohe, 2004; Rodríguez-Flores et al., 2019; Tuberoso, Bifulco, Caboni, Cottiglia, Cabras & Floris, 2009).

*Castanea* was the secondary pollen in most of the samples, with a mean value of 27.1% and a maximum value of 59.8%. The values of *Castanea* pollen were considered for honey typification according to their overrepresentation in the pollen spectra of honey. *Rubus* was also identified in all the samples, and *Cytisus* type was present in 97% of the samples. Both were considered accompanying pollens, together with *Eucalyptus*. The pollens of *Quercus, Salix*, *Echium*, *Frangula alnus*, *Prunus* type, *Trifolium* type and Poaceae were also observed in more than 50% of the samples. These pollen types are very common in the pollen spectra of honeys from this geographical region (Escuredo, Fernández-González & Seijo, 2012; Escuredo et al., 2013).

Regarding the different *Erica* species identified through the palynological analysis of the honey, the main were *E. umbellata*, *E. arborea* and *E. cinerea*. Additionally, a group of other species of *Erica*, named Other *Erica* species, was distinguished. Table 1 shows the values corresponding to the predominance of the species and the main secondary pollen types in these group of samples. The highest mean percentages were 39.7% for the type other *Erica* and 34.8% for *E. cinerea,* while the mean value for *E. umbellata* was 29.1% and for *E. arborea* was 28.2%. In all samples, *Castanea* and *Rubus* were present as secondary pollens. Furthermore, the *Cytisus* pollen type was found as a secondary pollen in samples with a predominance of *E. arborea* and *E. umbellata*. Finally, *Eucalyptus* was a secondary pollen in the honeys with a predominance of *E. cinerea*.

### 3.2. Volatile fraction of heather honeys

Honey has a high number of volatile organic compounds (VOC) found in very low concentrations (Jerković & Kuś, 2014). These may appear as more or less complex mixtures of different functional groups. Some of these compounds were identified, like alcohols, carbonyl compounds, carboxylic acids, esters, phenols, monoterpenes, norisoprenoids, and benzene derivatives, among others.

As a result of this study, 58 volatile compounds from heather honey samples were identified. Table 2 shows the retention time (RT), the calculated Linear Retention Index ($^{a}$LRI), and the Linear Retention Index Theoretical ($^{b}$LRI) obtained through the NIST Chemistry Web Book for each compound, and the relative concentration (%). Hotrienol was the main volatile compound detected in the SPME analysis for this honey type (Fig. 2), being represented in 82% of the samples. This compound was by far one of the compounds with the highest concentrations, with a mean value of 46%, reaching a maximum value of 69%. The presence of the compound hotrienol is common in floral scents and in several food products, including honey, and is typically

**Table 1**
Main pollen types according to the predominant *Erica* species.

| Main principal pollen | Secondary pollen | Mean (%) | Maximum (%) | Minimum (%) | SD |
|---|---|---|---|---|---|
| *E. arborea* $\bar{x}$ = 28.2%(17.5–36.9%) | *Castanea* | 14.1 | 46.6 | 0.0 | 17.4 |
| | *Cytisus* type | 10.1 | 17.4 | 0.3 | 7.3 |
| | *E. umbellata* | 9.8 | 20.4 | 0.0 | 9.6 |
| | *Rubus* | 8.8 | 19.8 | 2.8 | 6.1 |
| *E. cinerea* $\bar{x}$ = 34.7%(33–36.5%) | *Castanea* | 19.0 | 25.8 | 12.2 | 9.6 |
| | *Eucalyptus* | 22.9 | 29.2 | 16.6 | 8.9 |
| *E. umbellata* $\bar{x}$ = 29.1%(8.5–51.4%) | *Castanea* | 31.9 | 59.8 | 13.6 | 12.9 |
| | *Quercus* | 2.2 | 16.9 | 0.0 | 3.4 |
| | *Cytisus type* | 9.2 | 21.9 | 0.0 | 6.3 |
| | *E. arborea* | 4.0 | 17.9 | 0.2 | 4.2 |
| | *Rubus* | 10.4 | 30.7 | 0.8 | 7.8 |
| Other *Erica* $\bar{x}$ = 39.7%(27.4–48.6%) | *Castanea* | 23.4 | 37.7 | 8.0 | 14.9 |
| | *Rubus* | 13.4 | 27.0 | 4.4 | 12.0 |

$\bar{x}$: arithmetic average; SD: standard deviation; Secondary pollen: Pollen types that appeared between 15 and 45% in honey samples

**Table 2**
Main volatile compounds isolated by GC–MS (SPME) in heather honey samples.

| Volatile compounds | RT (min) | [a]LRI | [b]LRI | % Mean | Max | Min | SD |
|---|---|---|---|---|---|---|---|
| Phenylacetaldehyde | 11.1 | 1043 | 1046 | 11.3 | 37.0 | 1.5 | 10.6 |
| α-methylbenzyl alcohol | 11.9 | 1061 | 1046 | 5.2 | 7.1 | 3.1 | 1.6 |
| *cis*-Linalool oxide | 12.2 | 1068 | 1068 | 13.4 | 45.6 | 6.3 | 8.2 |
| *trans*-Linalool oxide | 12.9 | 1085 | 1086 | 5.1 | 15.1 | 2.2 | 3.4 |
| Methyl benzoate | 13.2 | 1092 | 1095 | 5.4 | 7.8 | 4.1 | 2.0 |
| Hotrienol | 13.9 | 1108 | | 45.7 | 68.9 | 14.2 | 14.1 |
| 2-Phenylethanol | 14.3 | 1117 | 1120 | 5.3 | 9.6 | 0.0 | 3.5 |
| Isophorone | 14.4 | 1119 | 1124 | 4.0 | 8.3 | 1.4 | 2.8 |
| 4-Oxoisophorone | 15.5 | 1143 | 1145 | 2.8 | 5.1 | 1.6 | 1.0 |
| 3,6-dihydro-4-methyl-2-(2-methyl-1-propenyl)–2H-pyran | 15.7 | 1147 | 1147 | 2.0 | 3.7 | 1.2 | 0.8 |
| Ethylbenzoate | 16.5 | 1165 | 1177 | 3.0 | 5.5 | 1.0 | 1.9 |
| Methyl 2-Phenylacetate | 16.9 | 1174 | 1245 | 4.7 | 6.1 | 3.3 | 2.0 |
| 2-methyl-2-Nonen-4-one, | 17.1 | 1178 | | 2.3 | 3.2 | 1.5 | 0.9 |
| Safranal | 17.9 | 1196 | 1197 | 4.4 | 7.9 | 1.6 | 2.4 |
| 2,6-dimethyl-3,7-octadiene-2,6-diol | 18.0 | 1198 | | 5.5 | 8.1 | 2.9 | 3.7 |
| Decanal | 18.3 | 1205 | 1208 | 2.7 | 8.3 | 0.8 | 2.8 |
| 3-Phenylfuran | 18.8 | 1216 | 1224 | 3.8 | 10.7 | 1.1 | 2.5 |
| 2,3-Dihydrobenzofuran | 19.2 | 1224 | 1237 | 5.4 | 10.4 | 2.2 | 3.1 |
| 1,4-dimethyl-2-octadecylcyclohexane | 19.7 | 1236 | | 0.8 | 1.0 | 0.7 | 0.2 |
| Ethylphenyl acetate | 19.8 | 1238 | 1245 | 1.2 | 1.5 | 1.0 | 0.3 |
| 4-methoxybenzaldehyde | 20.4 | 1251 | 1258 | 3.5 | 5.5 | 2.3 | 1.4 |
| Thymol | 22.3 | 1293 | 1295 | 3.8 | 14.7 | 0.9 | 4.0 |
| Decanoic acid, methyl ester | 23.6 | 1323 | 1322 | 4.6 | 6.5 | 2.7 | 2.7 |
| 4-methyl-1-Naphthalenol, | 23.8 | 1327 | | 1.6 | 2.1 | 0.9 | 0.5 |
| 1,1,5-trimethyl-1,2-dihydronaphthalene (TDN) | 24.5 | 1343 | 1354 | 8.0 | 21.2 | 0.6 | 7.4 |
| 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one | 25.7 | 1371 | | 2.1 | 5.4 | 0.1 | 1.5 |
| *n*-Decanoic acid | 26.7 | 1394 | 1394 | 4.3 | 5.7 | 3.5 | 1.2 |
| Ethyl decanoate | 26.8 | 1396 | 1403 | 2.2 | 2.8 | 1.7 | 0.6 |
| 1,1,6-Trimethyl-1,2-dihydronaphthalene | 26.8 | 1396 | | 2.4 | 5.3 | 0.8 | 1.5 |
| 1,6,6-Trimethyl-7-(3-oxo-but-1-enyl)-3,8-dioxatricyclo[5.1.0.0(2,4)]octan-5-one | 28.4 | 1434 | | 1.9 | 3.6 | 0.8 | 1.5 |
| 5-Methyl-2-phenyl-2-hexenal | 29.3 | 1455 | 1488 | 1.1 | 1.6 | 0.6 | 0.5 |
| γ-Decalactone | 29.6 | 1462 | 1490 | 5.7 | 13.0 | 1.6 | 3.0 |
| 4,6,10,10-tetramethyl-5-oxatricyclo[4.4.0.01,4]dec-2-en-7-ol | 29.8 | 1467 | | 5.7 | 10.8 | 1.8 | 3.6 |
| 4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one | 30.0 | 1471 | | 1.3 | 1.4 | 1.1 | 0.2 |
| δ-Decalactone | 30.2 | 1476 | | 3.2 | 12.1 | 0.6 | 2.4 |
| *cis*-2-hydroxy-1-(2-propenyl)-Cyclopentanecarboxylic acid, methyl ester | 30.2 | 1477 | | 6.8 | 9.2 | 4.5 | 3.3 |
| Ethyl isoallocholate | 30.6 | 1480 | | 1.2 | 1.4 | 1.0 | 0.3 |
| 1,3,5-trimethyl-2-octadecylcyclohexane (isomer 1) | 30.7 | 1489 | | 2.9 | 6.8 | 1.0 | 2.2 |
| Tetradecane | 30.9 | 1494 | | 1.8 | 2.2 | 1.5 | 0.5 |
| 2,3-Dimethyl-cyclohexa-1,3-diene | 31.3 | 1503 | | 4.1 | 8.6 | 1.2 | 1.8 |
| 6-Camphenol | 31.3 | 1503 | 1109 | 3.0 | 3.7 | 2.1 | 0.7 |
| Pentadecane | 31.4 | 1506 | | 1.0 | 1.3 | 0.8 | 0.3 |
| 1,3,5-Trimethyl-2-octadecylcyclohexane (isomer 2) | 31.5 | 1508 | | 2.5 | 4.6 | 1.4 | 1.8 |
| 1,7,7-Trimethylbicyclo [2,2,1]hept-5-en-2-ol | 31.5 | 1508 | | 7.1 | 8.5 | 5.0 | 1.5 |
| 3,7,7-Trimethyl-1-penta-1,3-dienyl-2-oxabicyclo[3.2.0]hept-3-ene | 32.3 | 1529 | | 5.7 | 26.2 | 1.1 | 4.9 |
| Megastigmatrienone (Isomer1) | 33.0 | 1546 | | 2.4 | 2.4 | 2.3 | 0.1 |
| Megastigmatrienone (Isomer2) | 33.7 | 1564 | | 7.4 | 10.5 | 2.9 | 2.9 |
| δ-Selinene | 34.4 | 1582 | | 0.8 | 1.0 | 0.5 | 0.2 |
| 4,4-dimethyl-6-ethyl-3,4-dihydrocoumarin | 34.4 | 1582 | | 1.0 | 1.3 | 0.7 | 0.3 |
| 4′-*t*-Butyl-2′,6′-dimethylacetophenone | 34.5 | 1584 | | 1.4 | 2.8 | 0.7 | 0.7 |
| Megastigmatrienone (isomer 3) | 34.9 | 1594 | | 1.6 | 1.7 | 1.5 | 0.1 |
| Megastigmatrienone (isomer 4) | 35.4 | 1607 | | 7.3 | 10.8 | 2.5 | 3.1 |
| 5-Isopropylidene-6-methyldeca-3,6,9-trien-2-one | 36.3 | 1631 | | 1.8 | 2.6 | 1.3 | 0.7 |
| α-Gurjunene | 36.3 | 1631 | 1404 | 3.8 | 7.5 | 1.9 | 3.2 |
| Tetratetracontane | 38.4 | 1687 | | 1.7 | 2.5 | 1.1 | 0.7 |
| Nonadecane (isomer 1) | 38.5 | 1689 | | 2.8 | 5.9 | 1.3 | 1.6 |
| Heptadecane | 39.0 | 1703 | | 1.9 | 2.1 | 1.8 | 0.2 |
| Nonadecane (isomer 2) | 44.6 | 1860 | | 2.0 | 3.1 | 1.1 | 0.7 |

RT: Retention time; %: indicates relative area (peak area relative to the total peak area); Max: Maximum; Min: Minimum; SD: Standard deviation; [a]LRI, linear retention index determined on a DB-5 MS fused silica column relative to a series of *n*-alkanes (C7–C40); [b]LRI: Linear retention index theoretical obtained through the NIST Chemistry Web Book, SRD 69.

found in large concentrations in other heather honeys (de la Fuente et al., 2005; Castro-Vázquez et al., 2009; Castro-Vázquez, Alañón, Gonzalez-Viñas & Pérez-Coello, 2012; Soria, Sanz & Martínez-Castro, 2009).

Other compounds were also found in relevant concentrations, including *cis*-linalool oxide and phenylacetaldehyde. Like hotrienol, phenylacetaldehyde has been also detected at high concentrations in heather honeys (de la Fuente et al., 2005; Castro-Vázquez et al., 2009, 2012; Tan, Wilkins, Holland & McGhie, 1989; Wolski, Tambor, Rybak-Chmielewska & Kedzia, 2006). The presence of both compounds in some honeys can be attributed to long-term storage or heat treatment. In these cases, and especially when the honey origin and production is unknown, the origin of these compounds is difficult to differentiate (Jerković & Kuś, 2014). Phenylacetaldehyde is an aromatic hydrocarbon that can be produced in honey from the amino acid phenylalanine by enzymatic catalysis (Jerković & Marijanović, 2010). Therefore, its content in unheated honey will depend on the content of phenylalanine, but also on the storage conditions (de la Fuente et al.

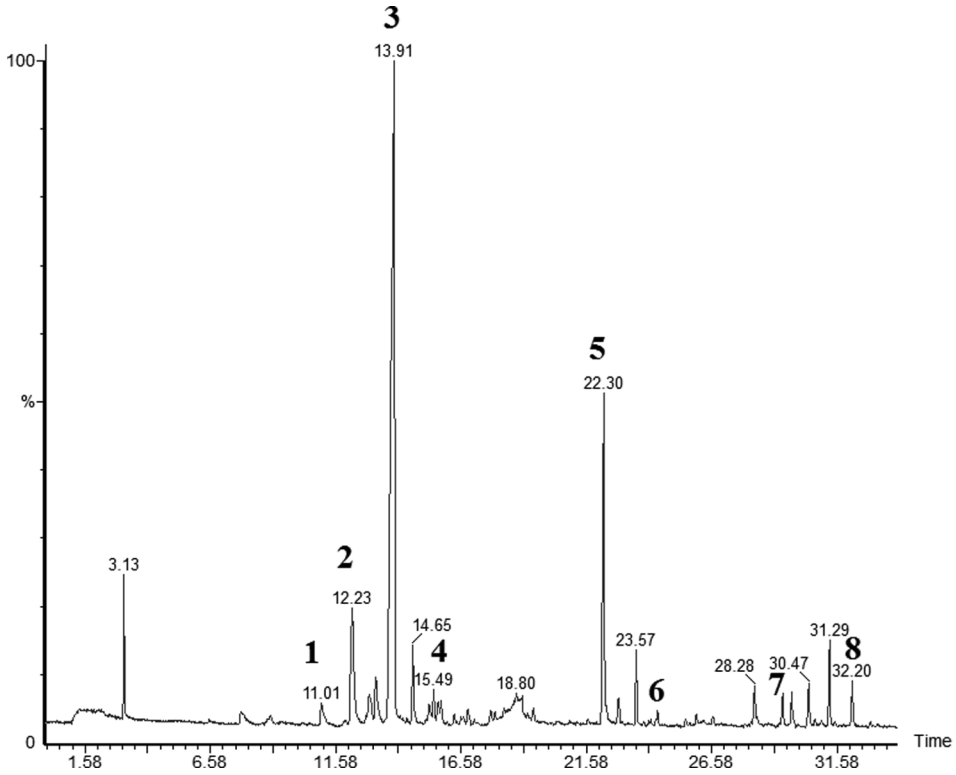


**Fig. 2.** GC–MS chromatogram of E. umbellata honey. **1:** phenylacetaldehyde; **2**: *cis*-Linalool oxide; **3**: hotrienol; **4**: 4-Oxoisophorone; **5**: thymol; **6**: TDN; **7**: 2(3H)-Furanone, 5-hexyldohydro-; **8**: 3,7,7-trimethyl-1 penta-1,3-diphenyl-2 oxabicyclo[3,2,0]hept-3-ene.

2005). Compounds such as *cis*-linalool oxide have been previously found in the volatile profile of heather honeys (da Silva et al., 2016; Castro-Vázquez et al., 2009; Radovic et al., 2001) or honeydew honeys (Jerković & Marijanović, 2010). On the other hand, some studies have linked the presence of these compounds with other sources rather than nectar, such as the hive atmospheres or the combustion of wood and biomass (Smith, Bromenshenk, Jones & Alnasseer, 2002) during beekeeping activity.

The volatile profile of honey is a fingerprint that can be used together with the melissopalynological analysis to determine its botanical origin. As reported earlier (Radovic et al., 2001; Jerković & Kuś, 2014), certain specific compounds are characteristic of honey from a specific floral source. In this regard, the frequency at which the compounds appear may be as important as their concentration, since they characterize the volatile profile and could be considered markers of heather honey. In the honeys under study, 3,7,7-trimethyl-1-penta-1,3-dienyl-2-oxabicyclo [3.2.0] hept-3-ene stood out, appearing in 97% of the samples. This is a product derived from the breakdown of carotenoids (Siems, Salerno, Crifò, Sommerburg & Wiswedel, 2009), with a probable plant origin, since it has been identified as a VOC in honeybush (*Cylclopia subternata*) (Roux, Cronje, Burger & Joubert, 2012). Degraded carotenoids, such as 3,5,5-trimethylcyclohex-2-ene derivatives, were also detected in heather honeys from New Zealand, and are considered possible markers for these honeys (Soria et al., 2009; Tan et al., 1989).

In addition to hotrienol, phenylacetaldehyde and *cis*-linalool oxide are worth mentioning due to their frequency of appearance, which surpasses 50%: 3-phenylfuran; δ-decalactone; γ-decalactone; 1,1,5-trimethyl-1,2-dihydronaphthalene (TDN) and 4-oxoisophorone. Furan compounds (Castro-Vázquez et al. 2009; Wolski et al., 2006) and lactone derivatives such as γ-decalactone (Karabagias, Maia, Karabagias, Gatzias, & Badeka, 2020), valerolactone (Guyot et al. 1999; Wolski et al., 2006), butyrolactone (Guyot et al. 1999), γ-butirrolactone (Radovic et al., 2001), and 4-oxoisophorone (de la Fuente et al., 2005; Soria et al., 2009; Wolski et al., 2006) have been previously described in heather honeys, and linked either with the presence of hexoses and pentoses or reactions of the shikimate pathway. On the other hand, naphthalene derivatives such as 1,1,5-trimethyl-1,2-dihydronapthalene or dihydro-trimethyl-naphthalene, although not previously associated with heather honey, have been observed in abundance in other honeys (Moniruzzaman, Rodríguez, Rodríguez-Cabo, Cela, Sulaiman & Gan, 2014; Soria et al., 2009).

Some of the identified volatile compounds, although detected at a very low frequency, at least in two samples, have previously been described as part of the volatile composition of the plant origin or as aromatic compounds in other food products. Some were also identified in the volatile fraction of heather honey (de la Fuente et al., 2005; Wolski et al., 2006; Radovic et al., 2001), including methyl benzoate; 2-phenylethanol; isophorone; ethylbenzoate; decanal; and 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one. Compounds such as δ-Selinene and 1,4-dimethyl-2-octadecylcyclohexane were found in concentrations less than or equal to 1%.

### 3.3. Volatile compounds according to the pollen profile

The organoleptic characteristics and consequently the volatile composition of honey are closely associated with its botanical source (Manyi-Loh et al. 2011). The dominance of the different species in the honey studied depends, additionally, on the area of origin and the harvest season. Thus, although honey is generally referred to as heather honey, different types of heather honey can occur based on different species of *Erica*. The presence of one *Erica* species is accompanied by other frequent pollen types in the samples. Each of these honey samples can present different or common VOCs, depending on this botanical profile. The relationship between the main VOCs and the main pollen types was assessed using principal component analysis (Fig. 3). Through this analysis, a small number of linear combinations of 24 variables (the volatile compounds γ-decalactone; 4-methoxybenzaldehyde; 3-phenylfuran; decanoic acid, methyl ester; 1,7,7-

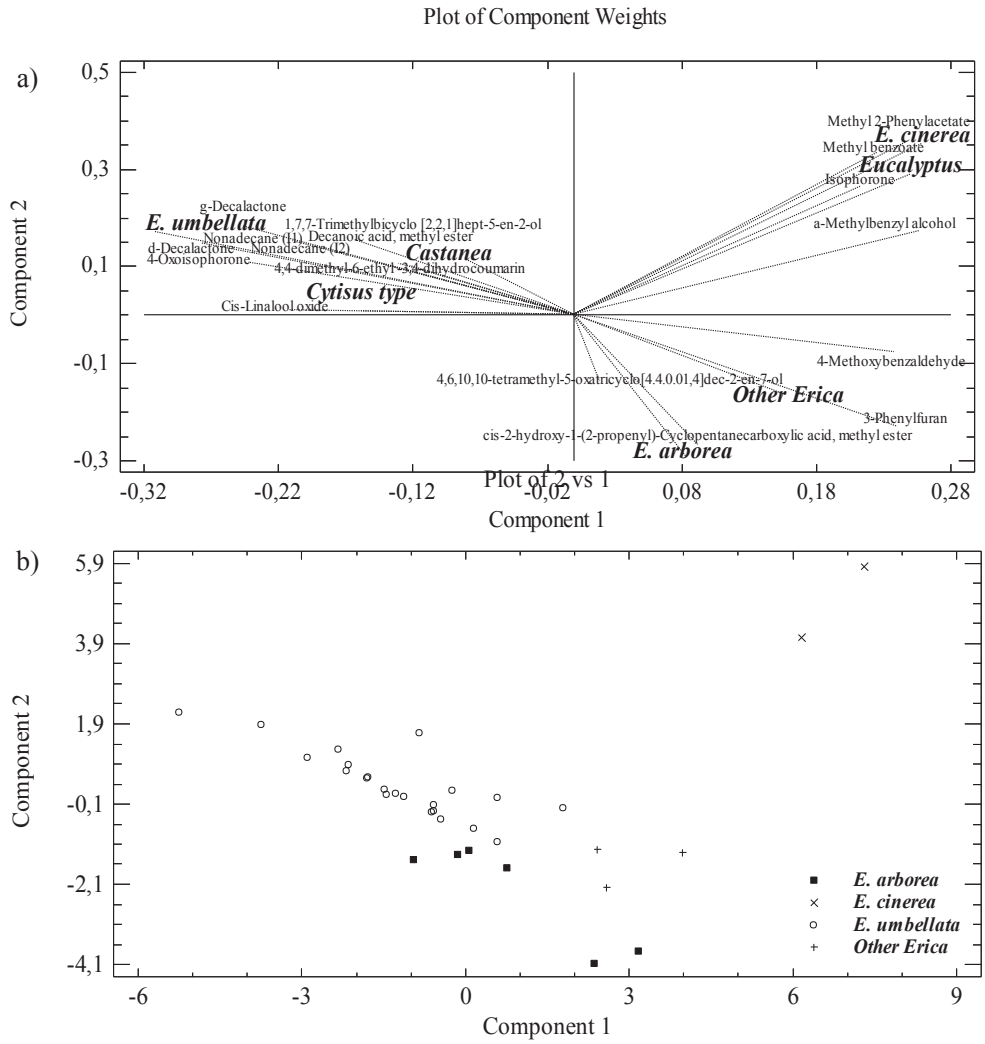


**Fig. 3.** The VOCs are represented in the upper figure according to the main pollen types. The graphical situation of the heather honey samples is shown in the figure below, depending on the predominance of the *Erica* species.

trimethylbicyclo [2,2,1]hept-5-en-2-ol; δ-decalactone; 4,4-dimethyl-6-ethyl-3,4-dihydrocoumarin; 4-oxoisophorone; 4,6,10,10-tetramethyl-5-oxatricyclo[4.4.0.01,4]dec-2-en-7-ol; α-methylbenzyl alcohol; methyl 2-phenylacetate; *cis*-Linalool oxide; *cis*-2-hydroxy-1-(2-propenyl)-Cyclopentanecarboxylic acid; methyl ester; isophorone; methyl benzoate; nonadecane (Isomer 1 and 2) and the main pollen types *E. arborea*; *E. umbellata*; *E. cinerea;* other *Erica; Castanea; Cytisus* type and *Eucalyptus*) were obtained, and these explain the variability of the data. In this case, seven components were separated. Together, they represent 81% of the variability in the original data. Fig. 3a shows the graphical representation of the first two components (43.3% of the variability). The most influential variables for Component One were the VOCs 3-phenylfuran; 4-oxoisophorone; γ-decalactone; δ-decalactone; and 4-oxoisophorone, and the pollen type *E. umbellata*. On the other hand, for Component Two they were the VOCs methyl 2-phenylacetate, methyl benzoate; and isophorone, and the pollen types *Eucayptus* and *E. cinerea.* At the other extreme of the same component, *cis*-2-hydroxy-1-(2-propenyl)-cyclopentanecarboxylic acid, methyl ester and 3-phenylfuran, and the pollen types *E. arborea* and other *Erica,* stood out.

The relationships among main pollen types and VOCs were checked using a Spearmańs rank correlation. Regarding the pollen type *E. umbellata* that appears in the space next to *Castanea* pollen, we found a positive correlation with nonadecane (Isomer 1 and 2) (Spearman's rank correlation (ρ) of 0.571 with a 99.0% confidence level ($P < 0.001$) and 0.393 with a 95.0% confidence level ($P < 0.05$), respectively); γ-decalactone ($\rho = 0.754$; $P < 0.001$); δ-decalactone ($\rho = 0.632$; $P < 0.001$); 1,7,7-trimethylbicyclo [2,2,1] hept-5-en-2-ol, and 4,4-dimethyl-6-ethyl −3,4-dihydrocoumarin ($\rho = 0.435$; $P < 0.05$) VOCs. *E. cinerea* showed positive correlations with α-methylbenzyl alcohol ($\rho = 0.364$; $P < 0.05$); methyl 2-phenylacetate ($\rho = 0.480$; $P < 0.001$); isophorone; and methyl benzoate ($\rho = 0.527$; $P < 0.001$), and the pollen type *Eucalyptus* ($\rho = 0.419$; $P < 0.01$), which presented the same correlations with the previous compounds. *E. arborea* had positive correlations with 4,6,10,10-tetramethyl-5-oxatricyclo[4.4.0.01,4]dec-2-en-7-ol ($\rho = 0.482$; $P < 0.001$) and *cis*-2-hydroxy-1-(2-propenyl)-cyclopentanecarboxylic acid, methyl ester ($\rho = 0.387$; $P < 0.001$). The group formed by other *Erica* showed positive correlations with 4-methoxybenzaldehyde ($\rho = 0.388$; $P < 0.05$) and 3-phenylfuran ($\rho = 0.455$; $P < 0.001$). Finally, the pollen type *Cytisus* was related with decanoic acid, methyl ester ($\rho = 0.399$; $P < 0.05$); 4-oxoisophorone ($\rho = 0.432$; $P < 0.05$); and *cis*-linalool oxide ($\rho = 0.589$; $P < 0.001$), along with *Eucalyptus.*

Fig. 3b represents the samples considering the values of the two first components, and depending on the predominance of the *Erica* species. It can be observed that samples were located according to the main pollen types and the VOCs with which they were correlated. Some of them, in which *E. umbellata* predominates, and others with a predominance of *E. arborea,* are very close. Both species can grow together, sharing the plant communities, therefore the nectar of both plants can be present in the same honey. On the other hand, the samples of *E.*

*cinerea* are further apart, showing the influence of other factors, such as the presence of *Eucalyptus*. As a result of the PCA, the pollen profile of samples helps us to understand the variability of the data, so this information plays a very important role in the study of VOCs in honey.

Some of the identified compounds in the samples have already been associated with certain botanical origins in honey. Nonadecane has been found in thyme honey from Greece (Alissandrakis, Tarantilis, Pappas, Harizanis & Polissiou, 2009), 2,3-dihydrobenzofuran in sage (*Salvia officinalis*) honey (Jerković, Mastelić & Marijanović, 2006), γ-decalactone in quince tree honey (Moreira, Trugo, Pietroluongo & De Maria, 2002) and in heather honey from Portugal (Karabagias et al. 2020), and thymol in *Thymus capitatus* honey (Kaškonienė & Venskutonis, 2010). The large amount of this latter compound may also point to the use of this substance by beekeepers as an acaricide in the sanitary control of the varroa mite (Bogdanov 2006). For *E. arborea* honey some authors, such as Guyot et al. (1999), have proposed some volatiles as markers; namely, 4-hydroxy-3-methoxybenzoate methyl ester (methyl vanillate); 4-methoxybenzaldehyde (*p*-anisaldehyde); and 4-methoxybenzoic acid (*p*-anisic acid). Other authors (Yang, Battesti, Paolini, Muselli, Tomi & Costa, 2012) have confirmed the presence of 4-propylanisol, *p*-anisaldehyde, benzaldehyde, and 3-furaldehyde as volatile compounds dominant in '*E. arborea* spring maquiś honeys. In this study, *p*-anisaldehyde was found as a volatile compound in six samples of *E. arborea*. However, this compound also appeared in *E. cinerea* and other *Erica* samples. Castro-Vázquez et al. (2009) and (2012) found this compound in heather honey, along with other compounds of the same nature such as guaiacol, propylanisole, and *p*-cresol. In the case of methyl 2-phenylacetate, although it has been identified in heather honey (Wolski et al. 2006), it has also been identified in the volatile profile of sage honey and in multifloral honey (Wolski et al., 2006; Kaškonienė & Venskutonis, 2010).

The number of shared VOCs in all samples was low (Table 3). From the identified compounds, seven were common to all the samples studied: phenylacetaldehyde; 3-phenylfuran; isophorone; 1,1,5-trimethyl-1,2-dihydronaphthalene; 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one; 1,1,6-trimethyl-1,2-dihydronaphthalene and 3,7,7-trimethyl-1-penta-1,3-dienyl-2-oxabicyclo[3.2.0]hept-3-ene. Table 3 also shows 20 VOCS that only appeared in heather honey samples with *E. umbellata;* γ-decalactone stood out, and one compound occurred in heather honey samples with *E. cinerea* and *E, arborea; cis*-2-hydroxy-1-(2-propenyl)-cyclopentanecarboxylic acid, methyl ester and methyl 2-phenylacetate, respectively.

Some of these compounds, such as phenylacetaldehyde; 3-phenylfuran; isophorone and 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one, have been attributed to heather honey in other studies (Boi, Llorens, Cortés, Lladó, & Llorens, 2013; Castro-Vázquez et al., 2009; de la Fuente et al., 2005; Guyot et al., 1999; Plutowska, Chmiel, Dymerski & Wardencki, 2011; Soria et al., 2009; Radovic et al., 2001; Tan et al., 1989; Wolski et al., 2006). In addition to these compounds, other VOCs identified in this study have been found in samples of heather honey of the *Erica* genus. The main compounds were hotrienol (Castro-Vázquez et al., 2009; de la Fuente et al., 2005; Karabagias et al., 2018; Soria et al., 2009) and linalool oxide (Boi et al., 2013; de la Fuente et al., 2005; Karabagias et al., 2018; Radovic et al., 2001; Soria et al., 2009; Wolski et al., 2006), and in lower amounts 2-phenylethanol (Castro-Vázquez et al., 2009; de la Fuente et al., 2005; Guyot et al., 1999; Soria et al., 2009; Tan et al., 1989); 4-methoxybenzaldehyde (*p*-anisaldehyde) (Castro-Vázquez et al., 2009; Guyot et al., 1999); 4-oxoisophorone (Boi et al., 2013; de la Fuente et al., 2005; Radovic et al., 2001; Soria et al., 2009; Tan et al., 1989; Wolski et al., 2006); decanal (Radovic et al., 2001; Wolski et al. 2006); decanoic acid (Guyot et al. 1999); ethylbenzoate (de la Fuente et al., 2005; Radovic et al., 2001); γ-decalactone (Karabagias et al. 2018); methyl benzoate (Tan et al.1989); and safranal (Boi et al. 2013). However, other compounds have been found in the literature, different from those in this study, which could demonstrate that the *Erica* species, together with the associated flora, determine the VOCs. Some of the most discussed are 1-hexanol (Castro-Vázquez et al., 2009; de la Fuente et al., 2005; Radovic et al., 2001); 2-Furanmethanol (Castro-Vázquez et al., 2009; de la Fuente et al., 2005; Wolski et al., 2006); 3-methyl-1-butanol (de la Fuente et al., 2005;

**Table 3**
VOCs present in samples regarding the main *Erica* species.

| Volatile compounds | *E. arborea* | | | *E. cinerea* | | | *E. umbellata* | | | Other *Erica* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Range | SD | Mean | Range | SD | Mean | Range | SD | Mean | Range | SD |
| 1,1,5-trimethyl-1,2-dihydronaphthalene (TDN) | 6.6 | 16.5–1.8 | 6.3 | 19.2 | 19.6–1.8 | 0.6 | 1.8 | 12.0–0.0 | 3.1 | 15.4 | 20.9–8.4 | 6.4 |
| Phenylacetaldehyde | 6.4 | 15.80–0.0 | 6.6 | 16.4 | 17.1–15.7 | 1.0 | 4.1 | 37.0–0.0 | 8.6 | 15.0 | 29.4–0.0 | 14.7 |
| Isophorone | 0.3 | 1.7–0.0 | 0.7 | 5.7 | 7.5–3.8 | 2.6 | 0.5 | 8.3–0.0 | 1.8 | 2.0 | 3.0–0.0 | 1.7 |
| 3,7,7-Trimethyl-1-penta-1,3-dienyl-2-oxabicyclo[3.2.0]hept-3-ene | 5.8 | 12.0–1.9 | 3.9 | 4.9 | 7.0–2.8 | 2.9 | 4.4 | 11.5–0.0 | 3.3 | 12.9 | 26.2–3.8 | 11.8 |
| 3-Phenylfuran | 4.3 | 10.7–0.0 | 4.4 | 3.5 | 3.6–3.4 | 0.2 | 2.1 | 7.2–0.0 | 2.0 | 5.2 | 7.4–3.7 | 1.9 |
| 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one | 1.7 | 5.4–0.0 | 2.2 | 2.6 | 2.8–2.5 | 0.2 | 0.2 | 2.5–0.0 | 0.6 | 1.1 | 2.2–0.0 | 1.1 |
| 1,1,6-Trimethyl-1,2-dihydronaphthalene | 1.6 | 5.3–0.0 | 2.5 | 1.4 | 1.4–1.3 | 0.1 | 0.1 | 2.5–0.0 | 0.5 | 1.4 | 2.0–0.8 | 0.6 |
| 1,4-dimethyl-2-octadecylcyclohexane | – | – | – | – | – | – | 0.07 | 1.0–0.0 | 0.2 | – | – | – |
| 1,7,7-Trimethylbicyclo [2,2,1]hept-5-en-2-ol | – | – | – | – | – | – | 1.3 | 8.5–0.0 | 2.8 | – | – | – |
| 2,3-Dihydrobenzofuran | – | – | – | – | – | – | 2.2 | 10.4–0.0 | 3.3 | – | – | – |
| 4,4-dimethyl-6-ethyl −3,4-dihydrocoumarin | – | – | – | – | – | – | 0.14 | 1.3–0.0 | 0.4 | – | – | – |
| 2,6-dimethyl-3,7-octadiene-2,6-diol | – | – | – | – | – | – | 0.5 | 8.1–0.0 | 1.8 | – | – | – |
| 4′-*t*-Butyl-2′,6′-dimethylacetophenone | – | – | – | – | – | – | 0.4 | 2.8–0.0 | 0.8 | – | – | – |
| 5-Isopropylidene-6-methyldeca-3,6,9-trien-2-one | – | – | – | – | – | – | 0.2 | 2.6–0.0 | 0.7 | – | – | – |
| 5-Methyl-2-phenyl-2-hexenal | – | – | – | – | – | – | 0.09 | 1.4–0.0 | 0.3 | – | – | – |
| Ethyl decanoate | – | – | – | – | – | – | 0.3 | 2.8–0.0 | 0.8 | – | – | – |
| Ethyl isoallocholate | – | – | – | – | – | – | 0.11 | 1.4–0.0 | 0.4 | – | – | – |
| γ-Decalactone | – | – | – | – | – | – | 4.6 | 13.0–0.0 | 3.5 | – | – | – |
| Heptadecane | – | – | – | – | – | – | 0.2 | 2.1–0.0 | 0.6 | – | – | – |
| Decanoic acid, methyl ester | – | – | – | – | – | – | 0.4 | 6.5–0.0 | 1.5 | – | – | – |
| *n*-Decanoic acid | – | – | – | – | – | – | 0.6 | 5.7–0.0 | 1.6 | – | – | – |
| Nonadecane (isomer 1) | – | – | – | – | – | – | 1.0 | 5.9–0.0 | 1.7 | – | – | – |
| Nonadecane (isomer 2) | – | – | – | – | – | – | 0.9 | 3.1–0.0 | 1.1 | – | – | – |
| Pentadecane | – | – | – | – | – | – | 0.09 | 1.3–0.0 | 0.3 | – | – | – |
| Tetradecane | – | – | – | – | – | – | 0.2 | 2.2–0.0 | 0.5 | – | – | – |
| Tetratetracontane | – | – | – | – | – | – | 0.2 | 2.5–0.0 | 0.6 | – | – | – |
| Thymol | – | – | – | – | – | – | 1.7 | 14.7–0.0 | 3.3 | – | – | – |
| *cis*-2-hydroxy-1-(2-propenyl)-Cyclopentanecarboxylic acid, methyl ester | 2.3 | 9.2–0.0 | 3.8 | – | – | – | – | – | – | – | – | – |
| Methyl 2-Phenylacetate | – | – | – | 4.3 | 5.6–3.0 | 1.9 | – | – | – | – | – | – |

Guyot et al., 1999; Radovic et al., 2001); 3-methyl-3-buten-1-ol (de la Fuente et al., 2005; Guyot et al., 1999; Soria et al., 2009; Radovic et al., 2001); acetoin (de la Fuente et al., 2005; Guyot et al., 1999; Radovic et al., 2001); benzaldehyde (Castro-Vázquez et al., 2009; de la Fuente et al., 2005; Guyot et al., 1999; Karabagias et al., 2018; Tan et al., 1989; Soria et al., 2009; Wolski et al., 2006); benzoic acid (Castro-Vázquez et al., 2009; Guyot et al., 1999; Plutowska et al., 2011); benzyl alcohol (Castro-Vázquez et al., 2009; de la Fuente et al., 2005; Radovic et al., 2001; Soria et al., 2009; Tan et al., 1989; Wolski et al. 2006); lilac aldehyde (Castro-Vázquez et al., 2009; de la Fuente et al., 2005and Wolski et al., 2006); and octanoic acid (Castro-Vázquez et al., 2009; Guyot et al., 1999; Wolski et al., 2006).

## 4. Conclusions

The analysis of volatile compounds using solid-phase microextraction (SPME) and GC–MS was successful in identifying the volatile profile of heather honey. The main compounds found in this honey were terpenoids, alcohols, benzene compounds, furan derivatives, and aldehydes. Hotrienol could be a clear marker for all heather honey, either due to its presence regardless of the *Erica* species or due to the high concentration observed on the volatile profile. Phenylacetaldehyde and *cis*-linalool oxide were also very abundant and frequent volatile compounds in all samples.

The correlations found between the pollen types of the palynological spectrum of the samples and the presence of certain volatile compounds reinforces the dependence of these compounds on the botanical origin of honey. In this sense, the dominance of the *Erica* species and the associated flora in the samples involved a specific volatile compound profile.

## CRediT authorship contribution statement

**M. Shantal Rodríguez-Flores:** Conceptualization, Methodology, Data curation, Writing - original draft, Software, Validation, Writing - review & editing. **Soraia I. Falcão:** Conceptualization, Methodology, Software, Validation, Writing - review & editing. **Olga Escuredo:** Supervision, Writing - review & editing. **M. Carmen Seijo:** Visualization, Investigation, Supervision, Writing - review & editing. **Miguel Vilas-Boas:** Conceptualization, Methodology, Visualization, Investigation, Writing - review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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