

Characterization of *Evodia rutaecarpa* (Juss) Benth honey: volatile profile, odor-active compounds and odor properties

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Abstract

BACKGROUND: Aroma is one of the most important quality criterion of different honeys and even defines their merchant value. The composition of volatile compounds, especially the characteristic odor-active compounds, contributes significantly to the aroma of honey. *Evodia rutaecarpa* (Juss) Benth honey (ERBH) is a special honey in China with unique flavor characteristics. However, no work in the literature has investigated the volatile compounds and characteristic odor-active compounds of ERBHs. Therefore, it is imperative to conduct systematic investigation into the volatile profile, odor-active compounds and odor properties of ERBHs.

RESULTS: The characteristic fingerprint of ERBHs was successfully constructed with 12 characteristic peaks and a similarity range of 0.785–0.975. In total, 297 volatile compounds were identified and relatively quantified by headspace solid-phase microextraction coupled with gas chromatography quadrupole time-of-flight mass spectrometry, of which 61 and 31 were identified as odor-active compounds by relative odor activity values and GC-olfactometry analysis, respectively, especially the common 22 odor-active compounds (*E*)- β -damascenone, phenethyl acetate, linalool, *cis*-linalool oxide (furanoid), octanal, hotrienol, *trans*-linalool oxide (furanoid), 4-oxoisophorone and eugenol, etc., contributed significantly to the aroma of ERBHs. The primary odor properties of ERBHs were floral, followed by fruity, herbaceous and woody aromas. The partial least-squares regression results showed that the odor-active compounds had good correlations with the odor properties.

CONCLUSION: Identifying the aroma differences of different honeys is of great importance. The present study provides a reliable theoretical basis for the quality and authenticity of ERBHs.

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Supporting information may be found in the online version of this article.

Keywords: *Evodia rutaecarpa* (Juss) Benth honey; characteristic fingerprint; volatile profile; odor-active compounds; odor properties; PLSR

INTRODUCTION

Honey, a sweet, flavorful and nutritious food, is collected by honeybees (*Apis cerana* or *Apis mellifera*) from nectar and/or honeydew.¹ The consumption of honey has increased dramatically as a result of its sensory characteristics and nutritional properties, and it is usually preferred by consumers for its sensory characteristics more than for its nutritional properties. Aroma is one of the most important quality criterion of honey and even defines its commercial value, mainly related to the composition of volatile compounds in honey, especially the characteristic odor-active compounds.^{1,2} Numerous work is underway aiming to identify the volatile compounds of honey as well as odor-active compounds and to characterize honey from a specific floral and/or geographic origin.^{3,4}

To date, more than 600 volatile compounds in low concentrations synthesized by various biosynthetic routes have been found in different honeys, classified according to their chemical classes as aldehydes, ketones, acids, alcohols, hydrocarbons, norisoprenoids, terpenes, benzene compounds, furan and pyran derivatives.^{5–9}

However, not all volatile compounds contribute to honey aroma because of different odor thresholds and interactions between compounds.¹⁰ Among them, more than 50 volatiles have been identified as odor-active compounds in different honeys.⁸ These odor-active compounds have been widely reported previously.^{10–12} (*E*)- β -damascenone and phenylacetaldehyde were identified in linden honey,¹³ rape honey¹ and apocynum honey¹²; benzyl alcohol

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(flowery) and phenylethyl alcohol (flowery) were identified in caju honey³ and citrus honey¹⁴; linalool and its derivatives, such as hotrienol, *cis*-linalool and *trans*-linalool oxides (furanoid), and lilac aldehydes were all been considered as important aroma compounds of Greek citrus honeys.¹⁵ Moreover, the aroma of honey varies considerably depending on the different nectar source plants, the maturity of the honey, the entomological origin of the bees, storage conditions, etc.^{6,16–18} Thus, the identification of odor-active compounds in honey contributes to the standardization of its quality, and is also of great significance in preventing fraudulent mislabeling of poor quality honey for its authenticity.^{19,20}

Currently, headspace solid-phase microextraction (HS-SPME) followed by gas chromatography with mass spectrometry (GC-MS),^{9,10,12,21,22} quadrupole time-of-flight mass spectrometry (GC-QTOF),²³ as well as comprehensive two-dimensional gas chromatography coupled to mass spectrometry (GC × GC-MS)^{8,24} and time-of-flight mass spectrometry (GC × GC-TOF-MS),²⁵ have been widely used for the identification of volatile profile and semi-volatile compounds in honeys. The odor activity values (OAVs), relative odor activity values (ROAVs) and GC-olfactometry (GC-O) can be used to select the key odor-active compounds affecting the aroma of the honey. There have been numerous studies using OAVs (OAVs ≥ 1) to investigate odor-active compounds in honeys,^{1,11,12,26} limited literature based on GC-O analysis^{10,14,21,27} and studies on ROAVs are scarce. Because the samples studied often contain tens or even hundreds of volatiles, the absolute quantification of them one by OAVs is laborious and costly, and so the relative percentage content of these compounds instead of their absolute concentration is an effective choice (i.e. ROAVs), in addition, GC-O can assist in the identification of some key aroma compounds missed in the OAVs/ROAVs, and the combination of them can make an objective evaluation and comprehensively identify key aroma compounds. Furthermore, tools of multivariate statistical techniques, such as principal component analysis, hierarchical clustering analysis and partial least-squares regression (PLSR), have been specifically performed to evaluate the contribution of the odor-active compounds in honey to its odor characteristic.^{7,21} However, no systematic study exists of the volatile compounds in honey based on the above-mentioned methods.

Evodia rutaecarpa (Juss) Benth honey (ERBH) is a special honey in China with unique flavor characteristics, produced by honeybees collecting nectar or honeydew from the nectar plant *Evodia rutaecarpa* (Juss.) Benth. (Rutaceae) (ERB). ERB is an important medicinal plant species with several varieties and is known as a traditional Chinese medicine for the treatment of postpartum hemorrhage, amenorrhea, abdominal pain, headache and dysentery.²⁸ Previous studies have shown that some of the volatile compounds in honey are plant derivatives,^{10,12,28} and it is worth investigating whether the volatile chemical composition of ERBH has the same composition and biological activities as the plant.

To the best of our knowledge, no work has yet been published in the literature to investigate volatile compounds and odor-active compounds of ERBHs. Therefore, the aims of this study were: (i) to characterize the volatile profiles in different ERBHs by HS-SPME/GC-QTOF analysis; (ii) to reveal the aroma profiles and odor properties of ERBHs based on ROAVs and GC-O analysis; and (iii) to explain the correlation between odor-active compounds and odor properties by PLSR.

MATERIALS AND METHODS

Honey samples and reagents

In total, eight ERBH samples, named FH1, FH2, FH3, FH4, FH5, FH6, JH1 and JH2, respectively, were collected directly under the guidance of local beekeepers from July to August, 2022. These samples were from two regions in China, six samples (FH1–FH6) were from Fujian Province, and the other two samples (JH1 and JH2) were from Jiangxi Province. Each sample (approximately 1 kg) was a part of a batch sample at least seven bee colonies. The ERBH samples were stored at 4 °C prior to analysis.

A mixture of *n*-alkanes (C7–C40) (Sigma-Aldrich, Shanghai, China) was employed for determining the linear retention indices (LRIs) of the compounds identified in the ERBH samples.

Analysis of volatile compounds

HS-SPME extraction conditions

HS-SPME was performed to extract volatile compounds from ERBH samples. The SPME arrow was divinylbenzene/carbon wide range/polydimethylsiloxane (1.1 mm × 120 μm) (PAL System, Zwingen, Switzerland). The honey sample (2.0 g) was weighed into a 20 mL SPME vial equipped with a Teflon-lined cap, then extracted in the SPME mode, with an incubation time of 20 min at 45 °C and an extraction time of 45 min, followed by the analysis conditions described below.

GC-QTOF analysis conditions

A 7890B series gas chromatography coupled with a 7200 series quadrupole time-of-flight mass spectrometry (GC-QTOF; Agilent, Santa Clara, CA, USA) equipped with an HP-5MS UI (30 m × 0.25 mm × 0.25 μm; Agilent) was performed to analyze the volatile compounds. Ultra-high purity helium was used as carrier gas at a flow rate of 1.0 mL min^{−1} and a solvent delay of 5 min. The GC oven temperature was initiated at 40 °C and held for 1 min, increased by 4 °C min^{−1} to 170 °C, then further increased to 260 °C by 20 °C min^{−1} and held for 3 min. Sample injection was conducted using the splitless mode with an injector temperature of 280 °C. The ion source and transfer line temperatures were set at 230 and 250 °C, respectively. The mass spectrometric ionization was performed in full scan mode with an EI voltage of 70 eV and TOF for MS was operated at 0.22 spectrum s^{−1} acquiring the mass range *m/z* 45–550.

Qualitative and quantitative of volatile compounds

The identification of volatile compounds was based on the comparison of mass spectra and LRIs matched to the NIST 17 library via MassHunter Workstation Unknowns Analysis, version 10.0 (Agilent). The candidate volatiles with mass spectrum match factors greater than 60 (maximum 100) and LRI offsets of plus or minus 20 units were identified as qualitative volatiles.

The volatile compounds of the ERBH samples were quantified based on the ratio between the peak areas of each compound to the sum of the areas of all qualitative volatile compounds after peak area normalization. The results were expressed as the mean ± SD (%) and compounds found in at least two experiments were confirmed for further analysis.

Identification of odor-active compounds

ROAV calculation

The ROAV was usually employed to evaluate the contribution degree of each volatile compound to the overall sample flavor in the field of food flavor research. For the main flavor compound

of the sample with $ROAV \geq 1$, the higher the ROAV value, the higher the contribution of the sample to the overall flavor, and $0.1 \leq ROA < 1$ was considered to be an important modifier of the overall flavor of the sample. In the present study, the ROAV method was used to screen and determine the odor-active compounds in ERBH samples, and was calculated by:

$$ROAV_i \approx 100 \times \frac{C_i}{C_{stan}} \times \frac{T_{stan}}{T_i}$$

where, C_i (%) and T_i ($\mu\text{g kg}^{-1}$) were the relative content of each volatile compound and its corresponding sensory threshold calculated in water, respectively; C_{stan} (%) and T_{stan} ($\mu\text{g kg}^{-1}$) were the relative content and corresponding sensory threshold

calculated in water of the compounds that contributed most to the overall aroma of the ERBH samples, respectively.

GC-O-MS analysis conditions

The GC-MS conditions were the same as for GC-QTOF, and the odor-active compounds of the ERBH samples were sniffed by three sensory evaluators using an odor detection port (Gerstel, Mülheim, Germany). Two replications of each ERBH samples were carried out for GC-O for each sensory evaluator.¹⁰ The evaluators sniffed at the outlet end of the olfactory detector, then described and recorded the aroma intensity (four levels: levels 1, 2, 3 and 4 represent weak, medium, strong and very strong, respectively), odor description and retention time. If two or more evaluators

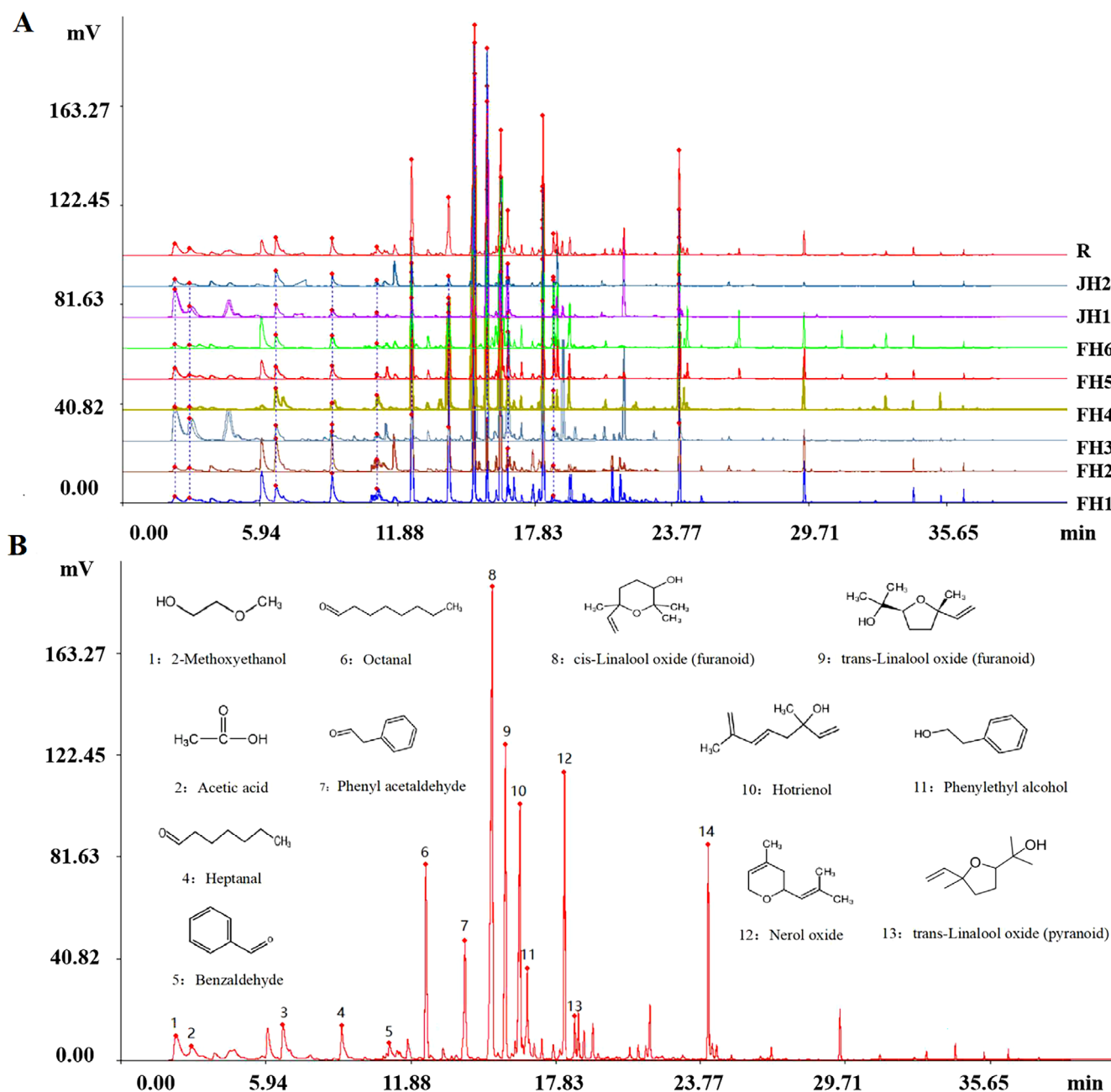


Figure 1. (A) Characteristic fingerprint and (B) identified 12 characteristic peaks of ERBHs.

sniffed similar odors at the same time period, the odors could be recognized as an odor-active compound.

Statistical analysis

The characteristic fingerprint of the ERBHs was established on basis of the total ion chromatogram (TIC) via the Similarity Evaluation System for Chromatographic Fingerprint of TCM (Version 2004 A, Chinese Pharmacopoeia Commission, China). Excel (Microsoft Corp., Redmond, WA, USA) was used for data processing, as well as pie, histogram and sunburst charts. An online website (<https://www.chiplot.online>) was used to draw heat maps and radar plot. PLSR plots were analyzed using The Unscrambler, version 10.4 (CAMO Software, Oslo, Norway).

RESULTS AND DISCUSSION

Characteristic fingerprints construction of ERBHs

A fingerprint is a characteristic profile or comprehensive pattern that represents the chemical composition of honey samples, which could provide the basis for authenticity, stability and standardization of honey products. Therefore, we first compared the area and retention time of each characteristic peak from the TIC of ERBH samples by HS-SPME/GC-QTOF analysis via the Similarity Evaluation System for Chromatographic Fingerprint of TCM (Version 2004 A). The volatile component chromatographic peaks of different ERBHs were matched and a control fingerprint R (as shown in Fig. 1A) with 77 chromatographic peaks and 14 characteristic peaks was automatically generated by adjusting the time window width, multi-point correction and spectral peak matching in the similarity evaluation system. It should be noted that peaks 3 and 14 were compared with the silicon-containing background peaks and were not analyzed in the present study.

The similarity values ranged from 0.785 to 0.975 (see Supporting information, Fig. S1), indicating a similar varieties of different

ERBHs. The retention time of the 12 characteristic peaks obtained from the standard fingerprint, as well as the library search results and the relative percentage contents of each volatile component, are shown in the Supporting information (Table S1). Twelve characteristic peaks were identified in the ERBHs, including 2-methoxyethanol, acetic acid, heptanal, benzaldehyde, octanal, phenyl acetaldehyde, *cis*-linalool oxide (furanoid), *trans*-linalool oxide (furanoid), hotrienol, phenylethyl alcohol, nerol oxide and *trans*-linalool oxide (pyranoid) (as marked in Fig. 1B).

The fingerprint of the volatile composition of honey referred to the ability to identify differences between samples such as different nectar plants, geographical origin or adulterated honey.^{29,30} A previous study established the chromatographic fingerprint of Polish goldenrod (*Solidago virgaurea* L.) honey, and found that hotrienol, nerol oxide and benzyl cinnamate could be considered as chemical markers specific to the botanical origin of goldenrod honey.³⁰ Overall, the establishment of characteristic fingerprints of different ERBH samples could help distinguish ERBHs from other varieties of honeys, which could also provide a basis for quality control of ERBHs.

Volatile compounds identified by HS-SPME/GC-QTOF

In total, 297 volatile compounds were identified in ERBHs by HS-SPME/GC-QTOF, which could be divided into eight categories according to their chemical structural properties, including acids, alcohols, aldehydes, esters, hydrocarbons, ketones, phenols and others (see Supporting information, Table S1). The main classes of volatile compounds were consistent with those analyzed by Karabagias *et al.*³¹ To better illustrate the contribution of each category of volatile compounds to the overall aroma of different ERBHs, the quantity percentage and total relative contents of each category of volatiles were counted and are presented in Fig. 2(A,B) (see also Supporting information, Table S1).

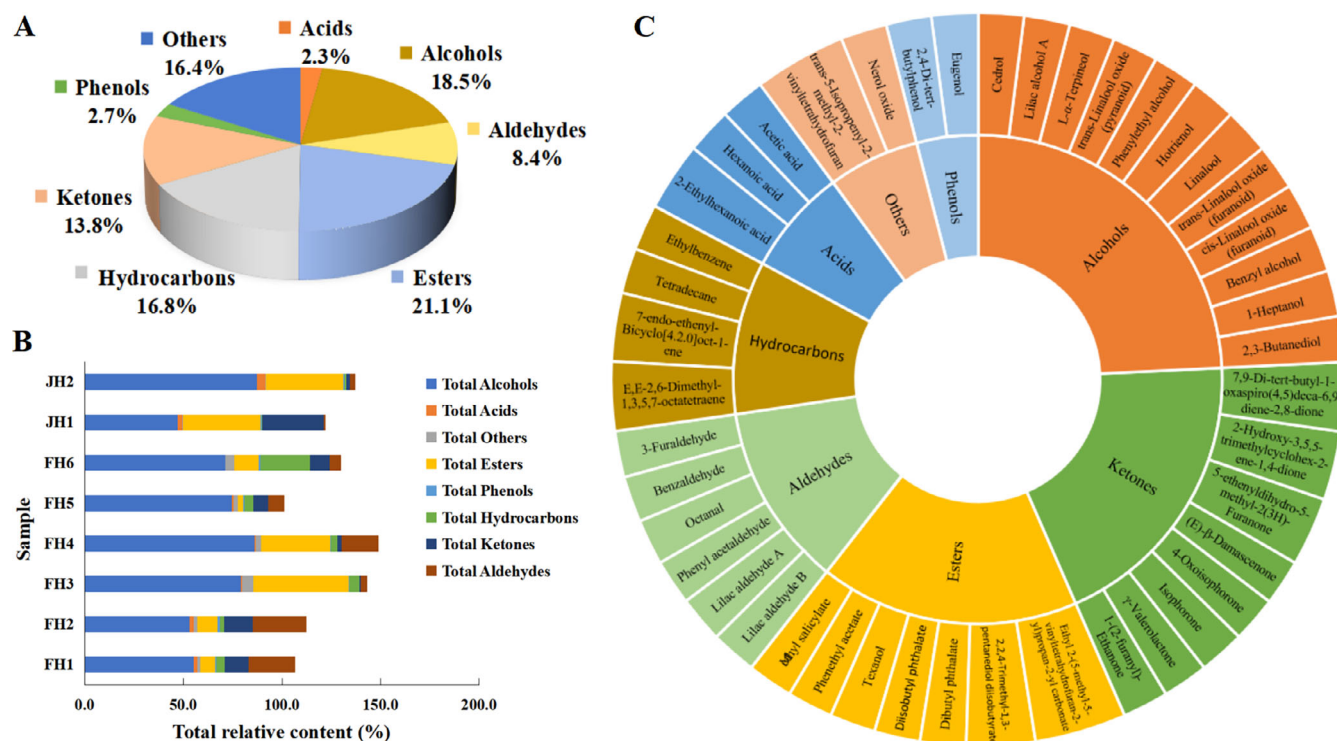


Figure 2. (A) Quantity percentage, (B) total relative contents of each category and (C) 44 common volatile compounds of ERBHs.

The presence of 63 esters (21.2%) was highlighted in different ERBHs, followed by 54 alcohols (18.2%), 50 hydrocarbons (16.8%), 41 ketones (13.8%), 49 others (some compounds containing nitrogen, sulfur, etc.) (16.5%), and, finally, 25 aldehydes (8.4%), 8 phenols (2.7%) and seven acids (2.4%) (Fig. 2A). Ester

compounds (especially those compounds with a retention time > 30 min) could be produced because of the increase in temperature at the time of injection, and these compounds were relatively low in content and contributed little or nothing to the aroma of ERBHs. Alcohols, aldehydes and ketones have been

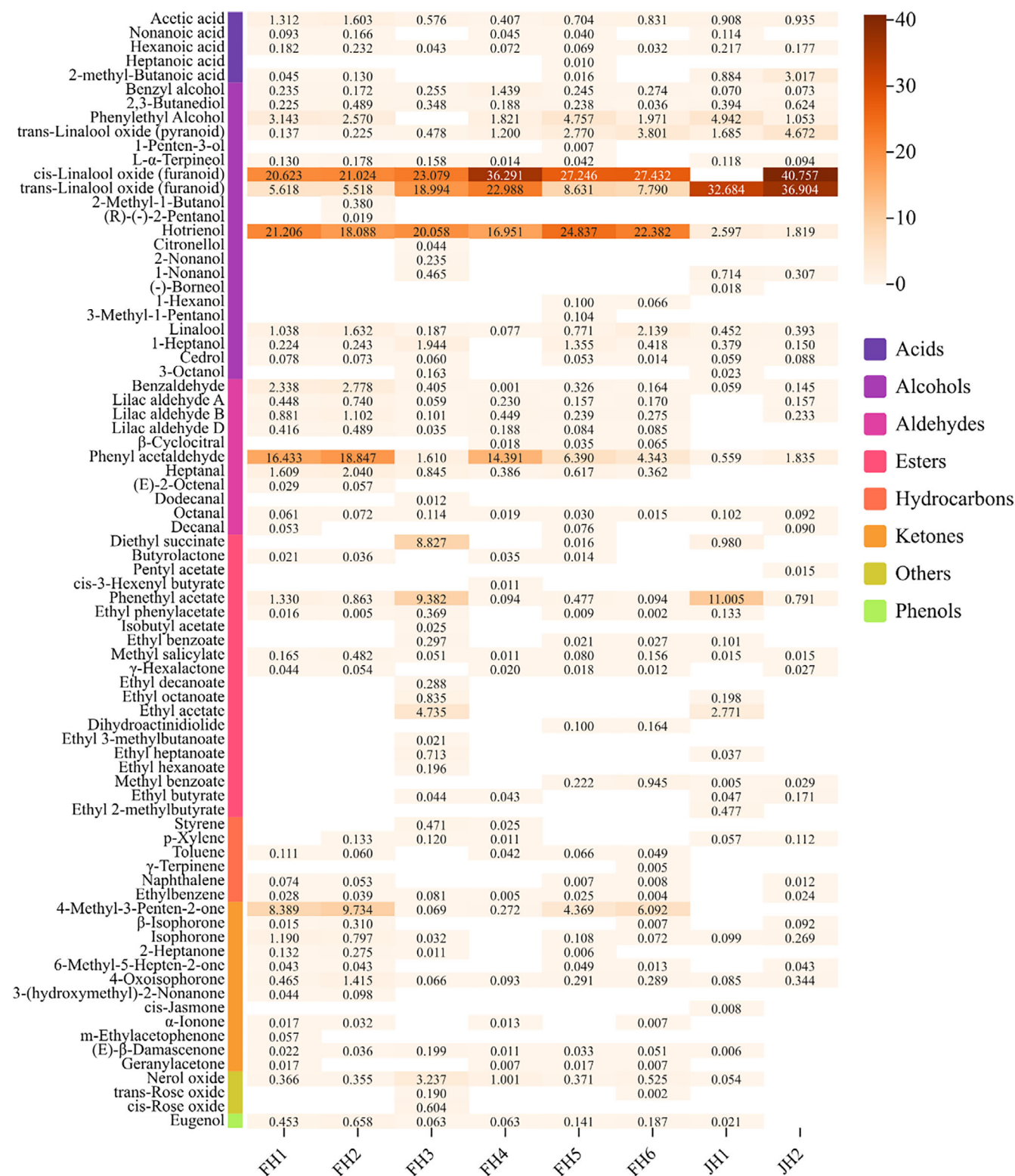


Figure 3. Heat map of the relative contents of 79 aroma compounds with sensory thresholds.

described as characteristic aromatic compounds of honey. Other classes of substances contribute to and modify the overall flavor of honey, and some substances can even be considered odor-active compounds in honey.

The total alcohols (47.1–87.2%) were the most abundant groups in terms of the total relative contents, followed by esters, aldehydes, ketones, hydrocarbons, others, acids and phenols (Fig. 2B). Meanwhile, the characteristic fingerprints and subsequent GC-O experiments showed that some compounds were present in all ERBHs, but are not listed in the Supporting information (Table S1) because of the low relative contents. Hence, we selected compounds present in at least seven samples for comparative analysis, and a total of 44 compounds were identified (Fig. 2C). Among them, acetic acid, 2,3-butanediol, benzyl alcohol, *cis*-linalool oxide (furanoid), *trans*-linalool oxide (furanoid), linalool, hotrienol, phenylethyl alcohol, benzaldehyde, phenyl acetaldehyde, octanal, γ -valerolactone, isophorone, 4-oxoisophorone, (*E*)- β -damascenone and eugenol have been widely reported previously,^{1,8–10,12,13,27,32–36} and were important contributors to the honey aroma. The other reported compound 2-methyl-butanoic acid was found in buckwheat honey,³⁷ hexanoic acid in lavender honey,³⁸ 1-heptanol in pomegranate honey,³⁹ L- α -terpineol and lilac alcohol A in Spanish citrus honeys,¹¹ lilac aldehydes B and D in orange honey and fir tree honey and honeydew,⁸ methyl salicylate in pine honey,¹⁹ phenethyl acetate in lavender honey,⁸ tetradecane in unifloral Greek citrus honey¹⁵ and *Quercus ilex* honey,⁹ and 5-ethenyl-2-methyl-2-(3H)-furanone in Robinia honey.⁴⁰

Most of the other compounds listed in the Supporting information (Table S1) have also been identified by researchers in studies of volatile compound profiles in different honeys. It should be noted, however, that not all of these compounds were present in all ERBHs, which may be related to the differences in nectar source plants, honey maturity, entomological origin of the bees, storage conditions, etc.^{6,16–18} and this requires further research.

Meanwhile, because literature about ERBHs was absent, any links with previous research could not be made. Thus, after clarifying the volatile profiles of ERBH samples, further research on the aroma profile of ERBHs was also crucial.

Odor-active compounds identified by ROAVs and GC-O

ROAV analysis

More than 600 volatile compounds have been identified and reported in honey,^{6–9} but only a small proportion of these can influence the overall aroma of honey, and such volatiles have been termed 'odor-active compounds'. To further determine the contribution of each volatile compound to the overall aroma of ERBHs, ROAV analysis was conducted.

Seventy-nine compounds with sensory thresholds (as listed in the Supporting information, Table S1) in water were selected as aroma compounds referring to previous literature about honey^{1,11,12,26,27} or other foods. The relative contents and their ROAVs of the 79 compounds are presented in Fig. 3 and Fig. 4(A), respectively. In total, 61 compounds with ROAV ≥ 1 were identified as the odor-active components, which were responsible for the aroma profile of the ERBHs, including 18 alcohols, 11 aldehydes, 13 esters, three others, two hydrocarbons, 12 ketones, one acid and one phenol. There are differences in the contribution of these odor-active compounds to different ERBHs. In the present study, the odor-active compounds in ERBHs with the highest ROAV included (*E*)- β -damascenone (ROAV: 2937.2–137 960.9), ethylbenzene (ROAV: 275.6–7019.4), phenethyl acetate (ROAV: 136.8–7171.6), 1-heptanol (ROAV: 43.4–898.5), linalool (ROAV: 11.3–415.9), *cis*-linalool oxide (furanoid) (ROAV = 100), octanal (ROAV: 23.9–225.8), hotrienol (ROAV: 14.3–299.1), eugenol (ROAV: 3.4–299.1), *trans*-linalool oxide (furanoid) (ROAV: 26.2–100.0) and cedrol (ROAV: 16.3–121.0), etc. In addition, 30 aroma compounds ($0.1 \leq \text{ROAV} < 1$) were found to have a modifying effect on the aroma quality of ERBHs (see Supporting information, Table S1). Among them, 13 common aroma compounds made important contributions to the aroma of some ERBH samples

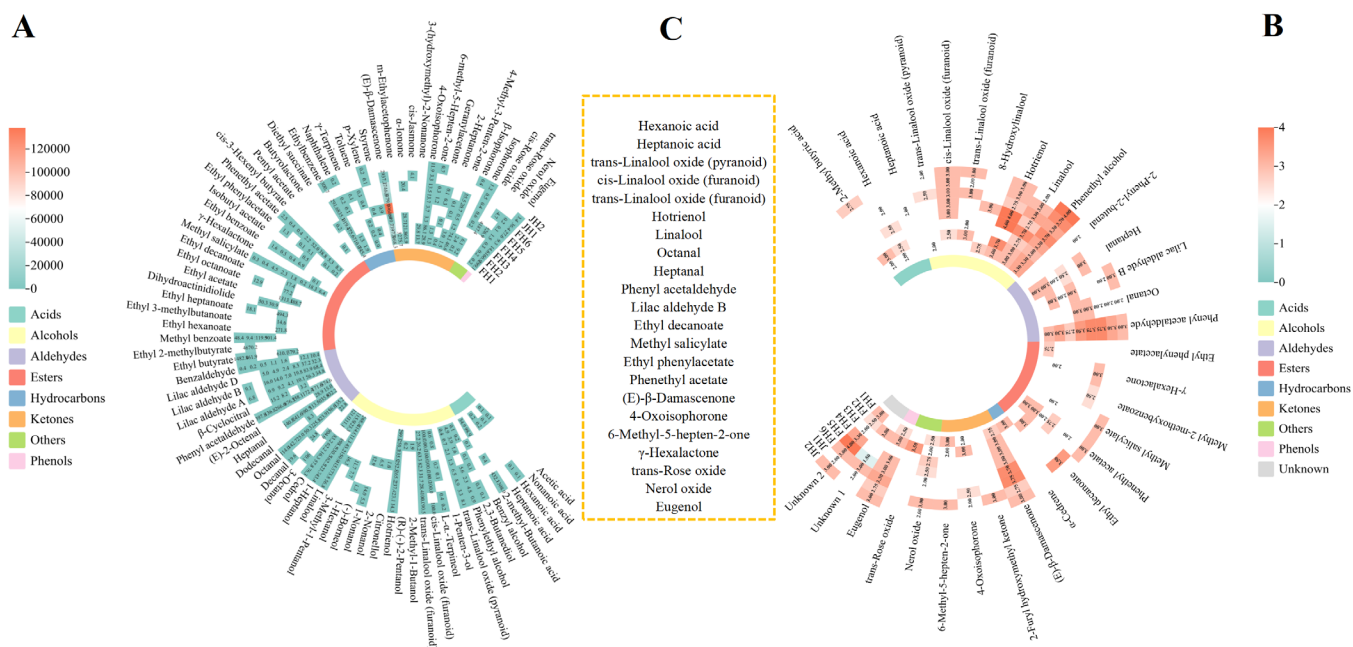


Figure 4. Heat map of the results of (A) relative odor activity values (ROAVs) analysis, (B) GC-olfactometry analysis and (B) the odor-active compounds common to both methods.

and also played a role in modifying and synergizing the aroma of other samples; for example, *trans*-linalool oxide (pyranoid) modified the aroma of FH1 and FH2 (ROAV was 0.4 and 0.7), at the same time as making an important contribution to the aroma of other ERBHs (ROAV = 1.3–8.9). Similar compounds include phenylethyl alcohol, benzaldehyde, methyl salicylate and phenethyl acetate, etc.

As mentioned above, most of the odor-active compounds in the present study were consistent with previous odor-active volatile compounds identified in other honeys.^{10–12} As stated previously, 2-methyl-butanoic acid (fermented, spoiled), the only key acid flavor compound in the present study, was also identified as an odor-active acid in citrus honey,¹⁴ odor-active alcohol compounds benzyl alcohol (flowery) and phenylethyl alcohol (flowery) were identified in caju honey³ and citrus honey¹⁴ as odor-active compounds, and the terpene compounds *cis*-linalool oxide (furanoid), *trans*-linalool

oxide (furanoid) and linalool, etc., were also identified as odor-active compounds in citrus honey.¹⁴ Linalool had also been reported as an odor-active compound in raspberry, rape and Alder buckthorn honeys, providing a flowery odor.¹⁰ Phenyl acetaldehyde, as odor-active aldehydes compounds, imparts a fruity, caramel odor to citrus honeys.^{11,14} Ethyl acetate, along with 2-butanone, 2-methylpropanal and α -pinene, could serve as the key indicators of the fluctuations the volatile composition of honeydew honey during storage at in-house conditions.¹⁸ Furthermore, phenyl acetaldehyde, linalool and lilac aldehydes have also been shown to contribute to the aroma of haze honey.⁴¹ Linalool and its derivatives, such as hotrienol, *cis*-linalool and *trans*-linalool oxides (pyranoid), and lilac aldehydes were all considered to be important aroma compounds of Greek citrus honeys.¹⁵ Important aroma compounds identified in the present study, such as (*E*)- β -damascenone, linalool,

Table 1. The aroma description and average aroma intensity of 31 olfactory aroma compounds of different ERBH samples

No.	Compound	Aroma description	Aroma type	Average aroma intensity								Identification ^b
				FH1	FH2	FH3	FH4	FH5	FH6	JH1	JH2	
1	2-Methyl butyric acid	Butter, cheese, fermented, sour	Microbiological	2	3	– ^a	–	–	–	–	2.75	MS, RI, R, O
2	Heptanoic acid	Rancid, sour, cheesy, sweet	Microbiological	2	2.5	–	2.5	–	2	–	–	MS, RI, O
3	Hexanoic acid	Acid, mushroom	Earthy	–	–	–	–	2	–	–	–	MS, RI, R, O
4	<i>trans</i> -linalool oxide (pyranoid)	Sweet, floral, earthy	Fruity	2	–	–	–	–	2.5	–	2	MS, RI, F, R, O
5	<i>cis</i> -linalool oxide (furanoid)	Floral, sweet, woody	Fruity	–	2.5	–	3	3	3	3	3	MS, RI, F, R, O
6	<i>trans</i> -linalool oxide (furanoid)	Sweet, floral	Fruity	–	3	2	–	–	3	2	3	MS, RI, F, R, O
7	8-Hydroxylinalool	Floral	Floral	2.75	–	–	–	3	–	–	–	MS, RI, O
8	Hotrienol	Floral, green, woody	Floral	3	3.7	–	4	4	2.75	3	3	MS, RI, F, R, O
9	Linalool	Citrus-like, floral	Fruity	3	3	2.75	3.7	2.75	3.3	3	2	MS, RI, F, R, O
10	Phenethyl alcohol	Rose-like, bitter, sweet	Floral	3.3	3.3	3	3.3	3.7	3.3	3.7	4	MS, RI, F, R, O
11	2-Phenyl-2-butenal	Cortex, sweet	Chemical	–	–	–	–	–	–	2	–	MS, RI, O
12	Heptanal	Pungent	Chemical	3	3	2	2.5	–	3	–	–	MS, RI, F, R, O
13	Lilac aldehyde B	Sweet, flowery	Floral	–	3	2	3	–	–	2	3	MS, RI, R, O
14	Octanal	Fruity	Fruity	–	–	–	3	3	2	2	2	MS, RI, F, R, O
15	Phenyl acetaldehyde	Green, sweet, floral	Fruity	3	3.3	2.75	3.5	3.75	3.75	3.3	3	MS, RI, R, O
16	Ethyl phenylacetate	Rose-like, honey	Floral	2.75	–	–	–	–	–	–	–	MS, RI, R, O
17	γ -Hexalactone	Coconut-like	Nutty	–	2	–	–	–	3	–	–	MS, RI, R, O
18	Methyl 2-methoxybenzoate	Herbal, floral, fruity	Herbaceous	–	–	–	–	–	2.5	–	–	MS, RI, O
19	Methyl salicylate	Mint-like	Herbaceous	–	–	2	–	–	–	–	3	MS, RI, R, O
20	Phenethyl acetate	Sweet, floral, rose-like	Floral	3	3	2	2.75	–	2	–	3	MS, RI, R, O
21	Ethyl decanoate	Waxy, coconut-like, Sweet	Nutty	–	3	–	–	–	–	3.5	–	MS, RI, R, O
22	α -Cedrene	Woody, sweet, fresh	Woody	3	–	–	–	–	–	–	–	MS, RI, O
23	(<i>E</i>)- β -damascenone	Apple-like, floral	Fruity	2.75	3	3	3	3.7	3.75	2.75	3	MS, RI, R, O
24	2-Furyl hydroxymethyl ketone	Stink	Chemical	–	–	–	–	–	–	3	–	MS, RI, O
25	4-Oxoisophorone	Musty, woody, sweet	Woody	2	3	–	–	–	2.5	2	–	MS, RI, R, O
26	6-Methyl-5-hepten-2-one	Citrus-like, green	Fruity	3	2	–	–	–	–	3	–	MS, RI, R, O
27	Nerol oxide	Green, herbal, floral, minty	Herbaceous	2.5	2	2.75	2.5	2	–	3	2	MS, RI, F, R, O
28	<i>trans</i> -rose oxide	Rose-like, floral, sweet	Floral	–	3.5	–	–	–	–	–	–	MS, RI, R, O
29	Eugenol	Clove-like, sweet	Herbaceous	2.5	3	–	3	3	3.3	2.75	3	MS, RI, R, O
30	Unknown 1	Floral, pungent	Chemical	–	3	–	–	1.5	3	2	–	O
31	Unknown 2	Sweet, floral	Floral	3	2.5	2	3.3	4	3	2	3	O

^a –, Not detectable.

^b Method of identification: MS, identified by mass spectra; RI, confirmed by retention index; F, characteristic fingerprint; R, ROAV analysis; O, GC-O analysis.

cis-linalool oxide (furanoid), benzaldehyde and phenyl acetaldehyde, etc., were also confirmed to be key aroma compounds in rape honey¹ and five unifloral honeys (apocynum, elaeagnus, lavender, jujube and amorpha) in Northwest China.¹²

Other compounds that had different aroma contributions to different ERBHs are listed in the Supporting information (Table S1). Because the present study aimed to analyze the aroma of ERBHs, the differences in aroma compounds caused by different sample sources were not carefully analyzed, and this will be investigated further in future work.

GC-O analysis

GC-O analysis is a powerful tool for the study of food flavors and it has been widely applied for aroma and flavor analysis of various

foods.⁴² To obtain a first insight into the ERBHs aroma, GC-O analysis was carried out as described above. Thirty one olfactory aroma compounds were identified, most of which were in line with previous studies,^{10,14,21,27,43} including one hydrocarbon, four ketones, five aldehydes, seven alcohols, six esters, one phenol, three acids, four others and two unknown compounds. The aroma description and average aroma intensity of 31 olfactory aroma compounds are presented in Table 1, and the start and end times and intensities are listed in the Supporting information (Table S2).

Among the 31 olfactory aroma compounds, the compounds presented in all ERBHs were (*E*)- β -damascenone (apple-like, floral), phenyl acetaldehyde (sweet, floral, rose-like), linalool (citrus-like, floral) and two unknown compounds (sweet). The first three compounds were identified in heather, raspberry, rape, buckthorn

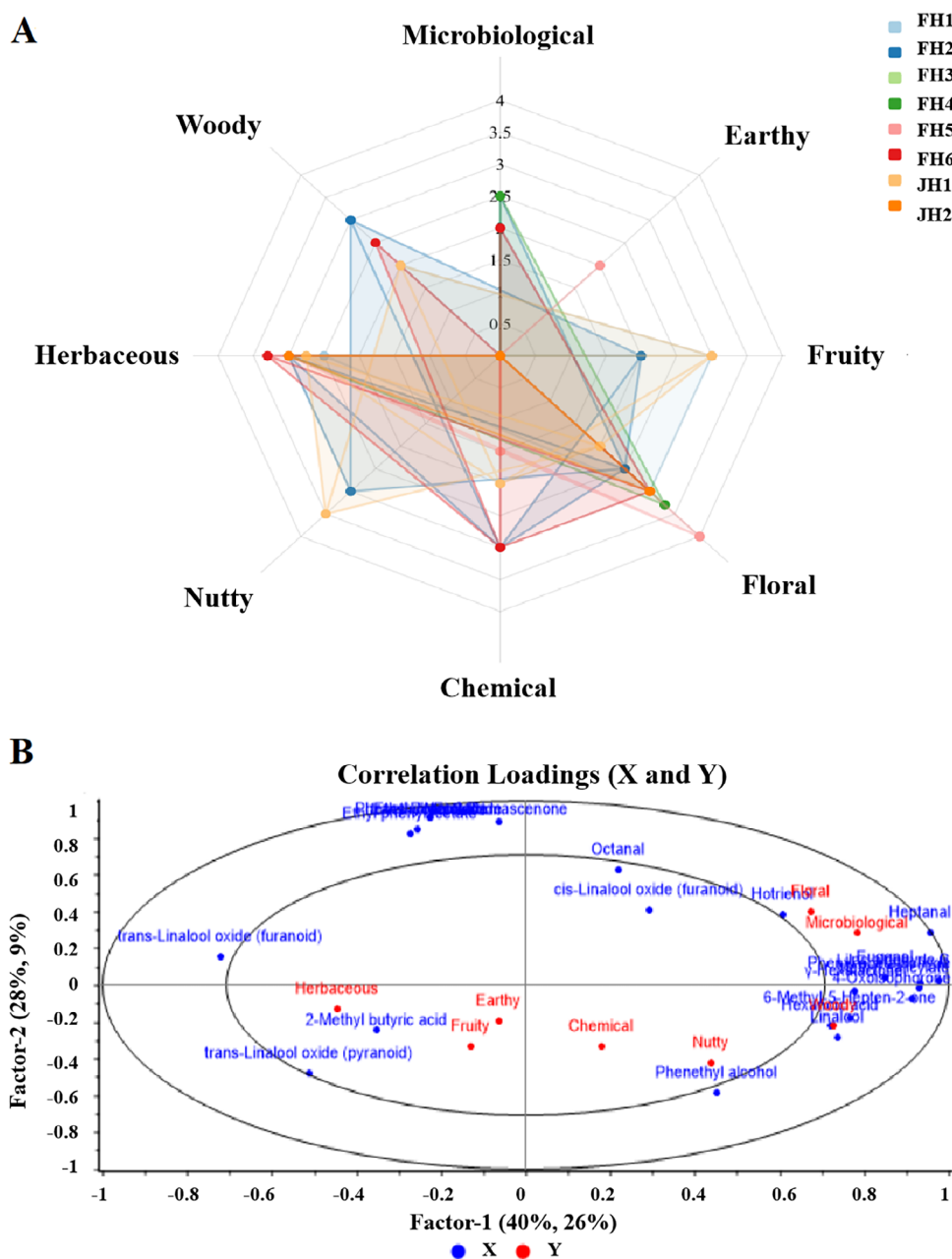


Figure 5. (A) Aroma profile of different ERBHs and (B) the correlation distribution of odor-active compounds and aroma characteristics.

honey and the blossoms of the four corresponding flowers,¹⁰ (*E*)- β -damascenone was identified in rape honey,¹ linalool in citrus honey¹⁴ and phenyl acetaldehyde in both types of honey, as well as finnish honeys,²¹ and (*E*)- β -damascenone and linalool were also found in the unifloral honey velame branco (*Croton heliotropiifolius* Kunth) by Costa et al.⁴³

Meanwhile, among the 31 olfactory aroma compounds (Fig. 4B), 22 were also present in the ROAV analysis (Fig. 4A), as shown in Fig. 4(C). In addition, the absence of some compounds from the ERBHs did not mean that these compounds were not detected by GC-O, and they may still be present in the ERBHs, but below their sensory threshold. Because the relevant sensory threshold of the compound was not found, the corresponding ROAV value was not calculated. For example, 8-hydroxylinalool was found in the GC-O analysis, but its ROAV could not be calculated because its sensory threshold was not found. In addition, the higher intensity compounds masked some lower intensity compounds as a result of co-elution, and some odor-active compounds were missed.^{10,21} Furthermore, the different precursor conditions based on the two methods are also possible reasons for the difference in compounds, with GC-O using air as the medium, and the calculation of ROAV was based on the sensory threshold of the compound in water.

Aroma profile evaluation

Referring to the previous standard descriptor categories for odor and flavor with sub-attributes reported by Kortessniemi et al.,²¹ we classified the GC-O analysis results of ERBHs into eight aroma types: microbiological, chemical, earthy, floral, fruity, herbaceous, nutty and woody. The specific aroma types of the 31 compounds are shown in Table 1, and the number of odor-active compounds for each aroma type was 2, 4, 1, 8, 8, 4, 2 and 2, respectively. The aroma profiles of the different ERBHs were then obtained from the average intensity values of odor-active compounds, as shown in Fig. 5(A). As can be seen from the radar plot, variations in the odor-active profile could be found because of the differences in the ERBH samples, although, as mentioned above, we did not analyze these differences in detail. Overall, based on the current analysis of aroma volatiles and odor-active profiles of ERBH samples, the odor characteristics of ERBHs could be described as sweet specialty honey with floral, especially rose-like aroma, accompanied by fruity, herbaceous, and woody aromas.

Additionally, an interesting finding was that α -cedrene was identified for the first time as an odor-active compound in ERBHs by GC-O analysis, suggesting that it may be a potential aroma compound in ERBHs, imparting an unique 'woody, sweet, fresh' aroma to ERBHs.

Correlation between odor-active compounds and sensory attributes by PLSR

To further investigate the effect of odor-active compounds on the overall aroma of ERBHs, PLSR was performed based on the results of ROAV, GC-O and aroma profile evaluation results. A relationship plot was then presented with 31 odor-active compounds as explanatory variables (*X*) and eight aroma attributes (microbiological, chemical, earthy, floral, fruity, herbaceous, nutty and woody) as dependent variables (*Y*) (Fig. 5B). The contribution of the *X* and *Y* of factor 1 and factor 2 was 40%, 26%, 28% and 9%, respectively. The outer and inner ellipses represented 100% and 50% of the explained variance, respectively.⁷ The aroma attributes and odor-active compounds located between the two ellipses can be well explained by the PLSR model. Variables in the same

quadrant had positive correlations, and the closer the distance, the greater the correlation.

Therefore, as can be seen from Fig. 5(B), the eight sensory attributes and 31 odor-active compounds were located between the two ellipses, meaning that these variables could be well explained by the PLSR model, and the 31 volatile compounds determined the aroma characteristics of the ERBH samples. The aroma attribute 'woody' was close to hexanoic acid (acid, mushroom), linalool (citrus-like, floral), 6-methyl-5-hepten-2-one (citrus-like, green) and γ -hexalactone (coconut-like), etc., and 'floral' and 'microbiological' were close to hotrienol (floral, green, woody), eugenol (clove-like, sweet) and heptanal (pungent), etc., indicating a correlation between the three sensory properties and these odor-active compounds; in particular, a strong correlation was observed between hexanoic acid and 'woody' aroma. Meanwhile, the five sensory attributes 'fruity', 'herbaceous', 'chemical', 'nutty' and 'earthy' of the ERBHs were located in the inner ellipse, indicating that the similarity between these attributes was large, and the contribution to the total variance of the PLSR model was not significant. In general, a good correlation was found between the evaluation of aroma profile and odor-active compounds of the ERBHs.

CONCLUSIONS

For the first time, the present study has systematically investigated the aroma profile, odor-active compounds and odor properties of ERBHs based on GC-QTOF, ROAVs, GC-O and PLSR analysis. The characteristic fingerprint of ERBHs was successfully constructed with 12 characteristic peaks and a similarity range of 0.785 to 0.975. In total, 297 aroma compounds were detected and relatively quantified, and 44 compounds were present in all ERBHs. Sixty-one compounds were identified as odor-active compounds with ROAV ≥ 1 . Thirty-one odor-active compounds were identified by GC-O analysis, and 22 compounds were consistent with ROAV results, including (*E*)- β -damascenone, phenethyl acetate, linalool, *cis*-linalool oxide (furanoid), octanal, hotrienol, *trans*-linalool oxide (furanoid), 4-oxoisophorone and eugenol, etc., which contributed significantly to the aroma of the ERBHs. The evaluation of the aroma profile revealed that the main odor properties of ERBHs were floral, followed by fruity, herbaceous and woody aromas. The PLSR results showed that the odor-active compounds had good correlations with the odor properties. These results provide a reliable theoretical basis for the quality and authenticity of ERBHs. Based on the results of the present study, further work should focus on identifying differences in nectar source plants, honey maturity, entomological origin of bees, storage conditions, etc., which will help to gain an in-depth understanding of the characteristic aroma profile of ERBHs.

AUTHOR CONTRIBUTIONS

HL, ZL and LC were responsible for sample collection. HL, MSh, MSo, DQ, WP and LC were responsible for conceptualization. HL and MSh were responsible for experiments. HL and ZL were responsible for software. HL, ZL, MSo and DQ were responsible for writing the original draft. WP and LC were responsible for project administration. LC was responsible for review and editing original draft. LC was responsible for funding acquisition.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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