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ORIGINAL RESEARCH ARTICLE

Volatile characterization of honey with dominance of *Bupleurum spinosum* pollen

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ABSTRACT

Moroccan honey with *Bupleurum spinosum* (Apiaceae/Umbelliferae) as the main pollen source is locally known as Zantaz honey. In the present work, the volatiles from 18 honeys of this type, were isolated by hydrodistillation and analysed by Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS), for volatiles quantification and identification. The percentage composition of the volatiles and pollen profiling was used to determine the relationship between the different samples by cluster analysis. Two poorly correlated clusters were defined in volatiles analysis. Cluster A, with 17 out of the 18 samples, included four subgroups dominated by straight-chain hydrocarbons and fatty acids in variable proportions. Cluster B included just one sample, with 1-phenyldodec-1-en-3-one as the main component. Mono- and sesquiterpene hydrocarbons, as well as oxygen-containing mono- and sesquiterpenes occurred always <2%. Two moderately correlated clusters were defined after pollen profiling cluster analysis. Pollen Cluster A included 17 samples having in common the presence of *B. spinosum* and *Populus* sp. pollen. The one sample from Cluster B was moderately correlated with Cluster A and showed the lowest percentage of *B. spinosum* pollen. Pollen volatiles analysis would be relevant in assessing the presence of putative pollen volatile marker compounds and the importance of pollen profile on honey volatiles.

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KEYWORDS
Bupleurum spinosum honey; Zantaz honey; volatiles; monofloral; unifloral

Introduction

Bupleurum spinosum Gouan is an Apiaceae (Umbelliferae) species, rich in terpenoids, polyacetylenes, flavonoids, and saponins (Barrero et al., 1998, 2000; Bencheraiet et al., 2012; Dahmoune et al., 2020). Many *Bupleurum* species have been used in several countries' traditional medicine for the treatment of inflammatory and auto-immune diseases (Yao et al., 2013).

Because of this broad range of biological activities, and because *Bupleurum* species are as well melliferous plants, honey with *Bupleurum* pollen predominance has also been the focus of several recent reports. Previous studies on Moroccan honey with *B. spinosum* as the main pollen source have addressed its melissopalynology, physicochemical and microbiological quality, as well as antioxidant, antibacterial, and antiproliferative activities assessment (Elamine, Imtara, et al., 2021b; Elamine, Lyoussi, et al., 2021; Elamine et al., 2018; Laaroussi et al., 2020). Locally known as Zantaz honey, among other designations, chemometric studies suggested that 40% is the monoflorality threshold of this dark-coloured honey (Elamine et al., 2019). Polyphenol characterization of Zantaz honey showed methyl syringate to be the most abundant compound, accounting for 40% to 58% of the total 18 polyphenols identified (Elamine, Lyoussi, et al., 2021). Although the individual polyphenols content was determined in this honey, no previous study addressed its volatiles composition.

As with any bee product, such as propolis or royal jelly, honey composition, namely their volatiles, vary with several factors prior to sampling, such as bee type, geographical origin, harvesting method, and local flora, among others. In addition, sampling and analysis procedures of honey volatiles, or from other bee products, can be performed in diverse ways (Derewiaka et al., 2021; Machado et al., 2020; Miguel

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& Figueiredo, 2017; Patrignani et al., 2018; Silva et al., 2021). Because volatiles isolation procedures may use humid-heat (Guo et al., 2022), such as in the hydrodistillation used in the current study, or a solvent of variable polarity (Osés et al., 2020) or a solvent-free system (such as headspace) (Zhu et al., 2022), the information gathered may be diverse and complementary, as has been shown for several monofloral honeys (Machado et al., 2021). Independently of the sampling procedure, honey volatiles are then analysed for the identification and quantification of their components. Gas chromatography (GC) and gas chromatography coupled mass spectrometry (GC-MS), or just GC-MS, are the techniques most frequently used for this purpose, sometimes the data being reported in percentages, in others in absolute amounts (Machado et al., 2020). Statistical analysis provides an understanding of the main influencing factors in honey properties and simplifies complicated problems. Chemometric analysis procedure is often used, including principal component analysis (PCA), partial least squares (PLS), orthogonal partial least-squares discrimination analysis (OPLS-DA), and cluster analysis (Machado et al., 2021; Silva et al., 2021; Zhu et al., 2022).

Honey volatiles are important in the honey industry, both from a customer and research perspective. If volatiles define honey aroma attributes, constituting one of the determining selection factors of consumers, they are also relevant in the characterization of monofloral (also called unifloral) honeys (Machado et al., 2020). In this context, the present work aimed at contributing to a better appreciation of *B. spinosum* honey by unraveling its volatile fraction, as well as assessing whether *B. spinosum* pollen dominance shapes the volatile composition in Zantaz honey.

Materials and methods

Bupleurum spinosum honey samples

In total 18 *Bupleurum spinosum* honeys (H1 to H18) were studied. Samples were obtained from Moroccan beekeepers as detailed in Elamine et al. (2018, 2019), Elamine, Imtara, et al. (2021). In short, the honey samples were obtained from beekeepers, in September 2014, at eight locations in Morocco, Supplementary File (S) Table S1, centrifuged, and stored in the dark at room temperature until use. Pollen profiling of each honey sample is provided in Table S1, adapted from Elamine et al. (2019). In brief, pollen qualitative and quantitative spectrum analysis followed the International Commission for Bee Botany (ICBB) guidelines as detailed in Louveaux et al. (1978). Pollen grains counting followed the criteria of a pollen species being considered dominant if it represented >45% of the pollen spectrum, secondary if it ranged between 16% and 45%, important minor pollen from 3 to 15%, and minor pollen when it was <3%. This indicated that 40% is the monoflorality threshold of Moroccan Zantaz honey (Elamine et al., 2019).

Bupleurum spinosum honey volatiles extraction

B. spinosum honey's volatiles were isolated by hydrodistillation for 1 h using a Clevenger-type apparatus according to the European Pharmacopoeia method (Council of Europe (2010). Given the volatiles low yield, they were recovered from the graduated tube of the Clevenger apparatus after rinsing with distilled *n*-pentane (*n*-pentane ≥99% purity, HPLC grade, is in lab distilled prior to use, to remove stabilizers that may contaminate the sample, particularly low volatiles yield samples) when the distillation procedure was over, and allowed to settle for about 10–15 min, as fully detailed in El-Guendouz et al. (2020). The mixture of distilled *n*-pentane and volatiles was recovered in an appropriate vial, and then concentrated to a minimum volume of about 100 µL, at room temperature under nitrogen flux, using a blow-down evaporator system. After isolation and until analysis, the honey volatiles extracts were stored at −20 °C in the dark.

Bupleurum spinosum honey volatiles composition analysis

Gas chromatography (GC)

Gas chromatographic analyses were performed using a Perkin Elmer Clarus 400 gas chromatograph equipped with two flame ionization detectors (FIDs), a data handling system, and a vaporizing injector port into which two columns of different polarities were installed: a DB-1 fused-silica column (polydimethylsiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 µm; J & W Scientific Inc., Rancho Cordova, CA, USA) and a DB-17HT fused-silica column [(50% phenyl)-methylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.15 µm; J & W Scientific Inc.]. The oven temperature was programmed, 45–175 °C, at 3 °C/min, subsequently at 15 °C/min up to 300 °C, and then held isothermal for 10 min; injector and detector temperatures, 280 °C and 300 °C, respectively; carrier gas, hydrogen, adjusted to a linear velocity of 30 cm/s (≈ 0.8 mL/min). The samples were injected using a split sampling technique, ratio 1:50. The volume of injection was 0.1 µL of a *n*-pentane-volatiles solution (1:1). The percentage composition of the volatiles was computed, by the normalization method from the GC peak areas, calculated as mean values of two injections, from each sample, without using the response factors, in accordance with ISO 7609.

Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS unit consisted of a Perkin Elmer Clarus 600 gas chromatograph, equipped with DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm; J & W Scientific, Inc.), and interfaced with a Perkin-Elmer 600 T mass spectrometer (software version 5.4.2.1617, Perkin Elmer, Shelton, CT, USA). Injector and oven temperatures were as above; transfer line temperature, 280 °C; ion source temperature, 220 °C; carrier gas, helium, adjusted to a linear velocity of 30 cm/s (≈ 0.8 mL/min); split ratio, 1:40; ionization energy, 70 eV; scan range, 40–300 u; scan time, 1 s. The identity of the components was assigned by comparison of their retention indices, calculated in accordance with ISO 7609, relative to C_8-C_{27} *n*-alkane indices and GC-MS spectra from a lab-made library, created with reference essential oils, laboratory-synthesized components, laboratory isolated compounds, and commercially available standards.

Statistical analysis

The percentage composition of honeys volatiles and pollen profile was used to determine the relationship between the different samples by cluster analysis using the Numerical Taxonomy Multivariate Analysis System (NTSYS PC software, version 2.2, Exeter Software) (Rohlf, 2000). For cluster analysis, the correlation coefficient was selected as a measure of similarity among all samples, and the Unweighted Pair Group Method with Arithmetical Averages (UPGMA) was used for cluster definition. The degree of correlation was evaluated according to Pestana and Gageiro (2000) in very high (0.90–1.0), high (0.70–0.89), moderate (0.40–0.69), low (0.20–0.39) and very low (<0.20).

According to Elamine et al. (2019), 40% is the monoflorality threshold of this honey, that is, the minimum *B. spinosum* pollen percentage needed to be present in honey to be considered a *Bupleurum* monofloral honey. Nevertheless, in this study, all samples were considered in both statistical analyses, regardless of the identified *B. spinosum* pollen percentage.

Results

Bupleurum spinosum honeys volatiles were obtained in a yield < 0.05% (v/w). Their chemical composition was a complex mixture in which up to fifty-two components were identified. The isolated volatiles are listed in Table 1, following their elution order on the DB-1 column, and arranged according to the lowest and the highest percentages found for each component in the two groups defined by agglomerative cluster analysis, Figure 1.

Despite the chemical diversity, the analyzed volatiles were predominantly straight-chain hydrocarbons or fatty acids-rich, or the main identified components were included in a chemical group designated "others", since the components grouped by their chemical structure were neither terpenes nor straight-chain hydrocarbons or fatty acids, as detailed in Supplementary File Figure S1. This "others" group was mainly composed by compounds derived from the primary metabolism, namely aldehydes and alcohols among others, Figure S1 (Supplementary File). One yet unidentified component (UI H) was obtained in relatively high percentages (≥5%) in one sample, Table 1. Data on the main mass spectra fragments of this component is provided in the footnote of Table 1.

Volatiles cluster analysis defined two poorly correlated clusters (Scorr < 0.3), Figure 1. Cluster A, with 17 out of the 18 samples analyzed, included four subgroups, A1, A2, A3, and A4, dominated by straight-chain hydrocarbons and fatty acids in variable proportions. A representative chromatogram of this group is provided in Figure S2. Cluster A1 was formed by six highly correlated samples (S_{corr} ≥ 0.94), Figure 1, being dominated by linoleic acid (22–30%), *n*-tricosane (16–23%), and *n*-pentacosane (11–20%). Cluster A2 also included six highly correlated samples (S_{corr} ≥ 0.94), with similar main components as Cluster A1, but with different ranges, namely, linoleic acid (31–52%), *n*-tricosane (12–17%) and *n*-pentacosane (10–15%). Cluster A3 comprised two samples, characterized by the dominance of *n*-tricosane (21–25%), and *n*-pentacosane (14–15%). Cluster A4, with three samples, was moderately correlated (S_{corr} ≥ 0.46) with clusters A1 to A3. Ethyl oleate (32–49%) was the dominant volatile component in the samples of Cluster A4. Cluster B included just one sample, with 1-phenyldodec-1-en-3-one (25%), *n*-tricosane (10%), and one yet unidentified component (14%) as main components. Mono- and sesquiterpene hydrocarbons, as well as oxygen-containing mono- and sesquiterpenes occurred always in percentages less than 2%.

Pollen profiling cluster analysis defined two moderately correlated clusters (S_{corr} < 0.5), Figure 2, Table 2. Cluster A, with 17 out of the 18 samples analyzed, included four subgroups, A1, A2, A3, and A4, having in common the presence of *B. spinosum* and *Populus* sp. pollen. Cluster A1 included eight highly correlated samples (S_{corr} ≥ 0.94), Figure 2, in which pollen of *B. spinosum*, *Populus* sp., and *Artocarpus altilis* was always present, despite the dominance of *B. spinosum* pollen (49–78%). Cluster A2 was formed by six highly correlated samples

Table 1. Minimum and maximum percentage range composition of the volatiles isolated by hydrodistillation from different *Bupleurum spinosum* honey samples.

		Bupleurum spinosum Honey Volatiles								
		Cluster A#								Cluster B
		Cluster A1		Cluster A2		Cluster A3		Cluster A4		
Components	RI	Min	Max	Min	Max	Min	Max	Min	Max	
n-Octane (C8)	800	t	0.8	t	0.4	0.6	2.8	0.2	1.2	0.8
2-Furfural	825	t	2.1	0.7	1.2	t	1.7	0.9	1.7	1.3
n-Hexanol	882	t	t	t	0.4	t	t	t	t	t
2-Acetyl furan	897	t	t	t	t	t	t	t	t	t
n-Nonane (C9)	900	t	t	t	0.2	t	t	t	t	t
Benzaldehyde	927	t	0.6	t	0.3	t	0.5	t	t	0.2
α-Pinene	930	t	t	t	0.2	t	t	t	t	0.2
5-Methyl furfural	938	t	t	t	t	t	1.0	t	t	0.2
Hexanoic acid	968	t	t	t	t	t	t	t	t	t
n-Octanal	973	t	t	t	t	t	t	t	t	t
Benzyl alcohol	1000	t	0.3	0.2	1.0	0.4	0.8	t	0.2	t
Benzene acetaldehyde	1002	t	0.3	0.2	1.1	0.4	5.7	t	0.1	t
1,8-Cineole	1005	t	0.9	t	0.3	t	t	t	0.3	0.7
Limonene	1009	t	0.9	t	0.2	t	t	t	0.2	t
n-Octanol	1045	t	t	t	t	t	t	t	t	t
6-Methyl-3,5-heptadien-2-one	1064	t	t	t	t	t	t	t	t	t
n-Nonanal	1073	t	0.1	t	0.2	0.1	0.3	t	t	0.1
Linalool	1074	t	0.1	t	0.2	0.1	0.3	t	t	0.1
Isophorone	1074	t	t	t	t	t	t	t	t	t
n-Undecane (C11)	1100	t	t	t	t	t	t	t	t	t
4-Keto-isophorone	1101	t	t	t	t	t	t	t	t	t
Octanoic acid	1149	t	t	t	t	t	t	t	t	t
α-Terpineol	1159	t	0.9	t	0.8	t	t	t	0.4	0.4
n-Decanal	1180	t	t	t	t	t	t	t	t	t
2,3,5-Trimethyl phenol	1252	t	t	t	t	t	t	t	t	t
Nonanoic acid	1263	t	0.2	t	t	t	t	t	t	0.7
2-Undecanone	1275	t	t	t	t	t	t	t	t	t
3,4,5-Trimethyl phenol	1277	t	0.3	t	0.2	t	t	t	0.5	0.1
n-Tridecane (C13)	1300	t	t	t	t	t	t	t	t	t
1,2-Dihydro-1,1,6-trimethyl-naphthalene (= Dehydro-*ar*-ionene)	1328	t	1.7	t	1.1	t	0.6	t	0.8	1.4
trans-β-Damascenone	1372	t	t	t	t	t	t	t	t	t
β-Bourbonene	1379	t	t	t	t	t	t	t	t	t
β-Caryophyllene	1414	t	0.7	t	t	t	t	t	t	t
6-Methyl-6-(5-methylfuran-2-yl)-heptan-2-one *	1420	1.1	6.4	1.2	6.5	t	1.1	1.2	2.7	8.8
n-Pentadecane (C15)	1500	t	0.2	t	0.2	t	0.3	t	t	0.1
Viridiflorol	1569	t	0.2	t	t	t	t	t	t	0.2
α-Bisabolol	1656	t	t	t	t	t	t	t	t	0.3
n-Heptadecane (C17)	1700	t	0.3	t	0.3	0.3	0.7	t	t	0.1
n-Nonadecane (C19)	1900	t	1.0	0.2	1.2	1.4	2.0	0.2	0.3	0.2
n-Octadecanal	2008	0.5	3.1	0.6	6.7	0.6	1.2	1.4	3.4	t
n-Octadecanol	2095	0.7	1.3	0.1	1.2	1.3	2.0	0.8	1.3	0.9
Methyl oleate	2096	t	2.6	0.6	2.7	t	0.4	1.3	1.8	3.2
n-Heneicosane (C21)	2100	1.2	2.9	1.1	3.1	3.8	4.5	0.5	0.9	0.9
Oleic acid	2101	2.5	6.5	2.3	7.4	0.5	4.1	3.4	3.9	6.5
Linoleic acid	2108	22.0	29.7	30.5	51.5	6.1	12.9	9.0	29.0	4.3
Ethyl oleate	2137	t	8.5	0.4	9.2	t	7.5	32.0	48.5	1.9
1-Phenyldodec-1-en-3-one *	2139	t	0.7	t	0.1	t	0.6	0.2	0.6	24.5
UI H	2142	0.5	2.0	t	3.0	1.5	3.8	t	1.5	14.2
(Z)-9-Tricosene *	2287	0.7	2.4	1.0	1.9	1.9	3.0	0.5	0.9	1.1
n-Tricosane (C23)	2300	16.4	23.1	12.3	16.5	21.1	24.6	7.6	13.2	10.0
n-Tetracosane (C24)	2400	0.6	2.1	0.5	1.4	0.7	2.0	0.3	0.5	0.1
n-Pentacosane (C25)	2500	10.7	19.8	9.9	15.2	14.3	14.7	5.4	7.3	5.1
n-Heptacosane (C27)	2700	2.5	7.9	2.5	6.3	2.9	3.7	1.4	3.0	1.2
% Identification		86.0	93.2	87.7	94.0	74.5	81.0	90.8	94.3	75.6

#For samples grouped on each of the clusters and subclusters see Figure 1. RI: In the lab calculated Retention Index relative to C_8-C_{27} *n*-alkanes on the DB-1column. UI: Unidentified compound. *Identification based on mass spectra only. t: trace (<0.05%). Unidentified compound MS (EI, 70 eV) *m/z* (Intensity ≥10%): *UI H*: 40 (15), 42 (16), 54 (24), 57 (12), 69 (39), 77 (17), 83 (11), 97 (11), 105 (100), 120 (17), 147 (55), 161 (32), 162 (79), 175 (12), 256 (9).

($S_{corr} \geq 0.94$), Figure 2, where *B. spinosum* pollen was also dominant (46–65%), *Populus* sp. pollen was also present in all samples, as well as *Cytisus* sp. pollen. Cluster A3, with two samples with high correlation ($S_{corr} \geq 0.84$), was still characterized by *B. spinosum* pollen dominance (30–37%), although in more balanced amounts with *Populus* sp. pollen (21–22%). In addition to *B. spinosum* and *Populus* sp. pollen, the two samples of this Cluster also showed the largest diversity of pollen types in common, namely *Euphorbia* sp., *Cytisus* sp., *Quercus* sp., *Salvia* sp., *Phytolacca americana*, and *Eragrostis pilosa*. Cluster A4, with one sample, was moderately correlated ($S_{corr} \geq 0.50$) with clusters A1 to A3. In this Cluster, *B. spinosum* pollen (22%) was within the range of other dominant pollens, those of *A. altilis*

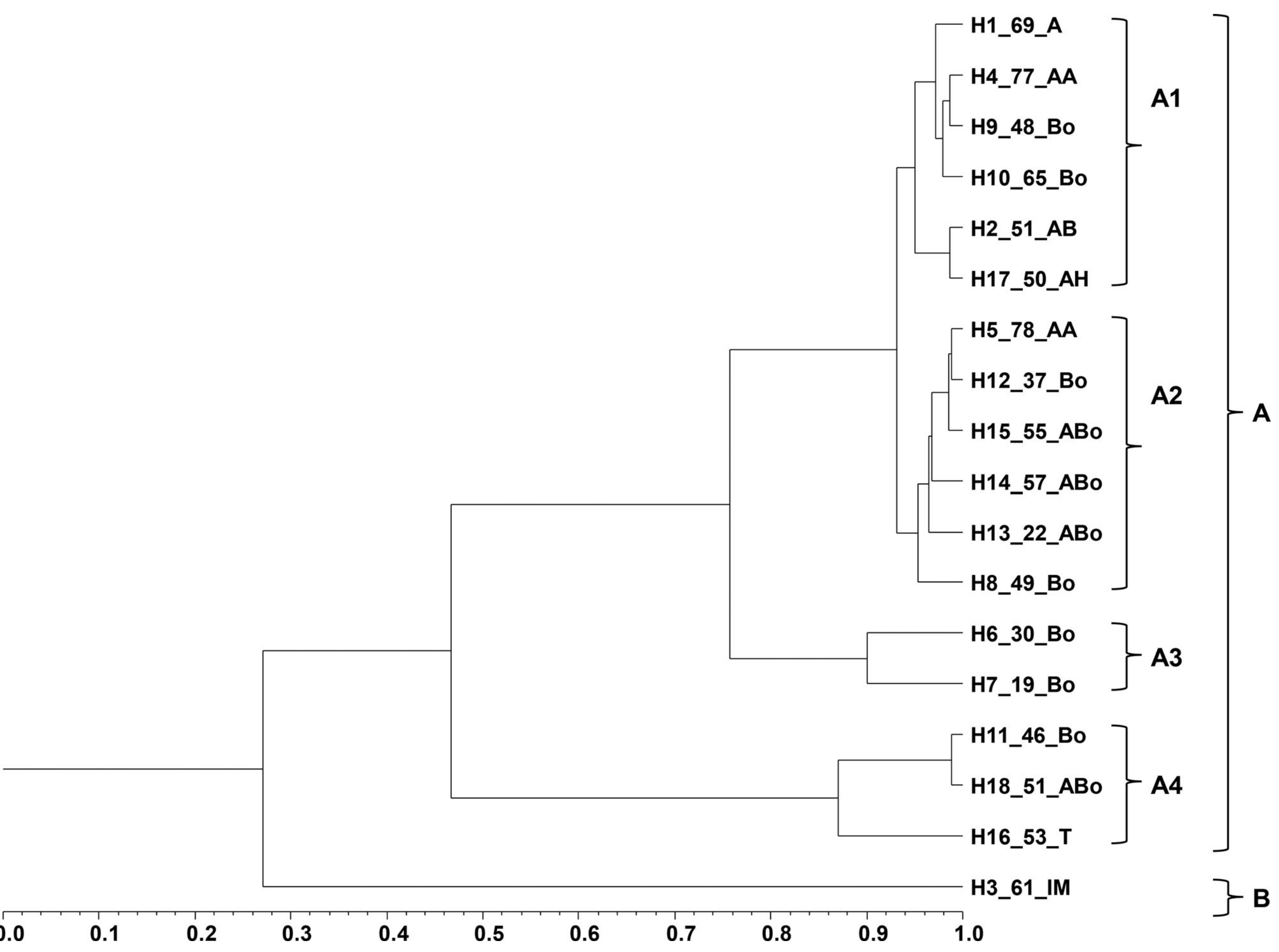

Figure 1. Dendrogram obtained by cluster analysis of the percentage composition of the volatiles isolated from *Bupleurum spinosum* honey samples evaluated, based on correlation and using unweighted pair-group method with arithmetic average (UPGMA). H: honey samples H1 to H18, with reference to *B. spinosum* pollen dominance after the underscore. For collection site codes, *vide* Table S1 (Supplementary File).

(28%) and *E. pilosa* (26%). Also with just one sample, Cluster B was moderately correlated ($S_{corr} \geq 0.46$) with Cluster A, Figure 2. Cluster B showed not only 10% of unidentified pollen but the lowest percentage of *B. spinosum* pollen (19%) and the dominance of *Cytisus* sp. pollen (46%), Table 2.

Discussion

In this study, hydrodistillation was selected as *B. spinosum* honey volatiles isolation procedure, as this is a common methodology of essential oils and different matrices volatiles extraction (Rubiolo et al., 2010), as well as from bee products (Machado et al., 2020; Miguel & Figueiredo, 2017).

Previous studies by Elamine, Lyoussi, et al. (2021) showed methyl syringate as the dominant phenolic compound in six methanol-extracted honeys. Its presence in the volatile fractions, obtained in the present study by hydrodistillation, could only be detected in below trace amounts in some samples, because of the occurrence of the specific marker fragmentation pattern.

B. spinosum honeys volatiles dominance in straight-chain hydrocarbons, fatty acids, or in the group designated "others", could not be correlated to the collection site, Figure 1 and Figure S1 (Supplementary File). Clusters with the largest number of samples, such as Cluster A1 and A2, gathered samples from several locations (Cluster A1, five, and Cluster A2, three of the eight collection sites). Also, when more than one sample was collected from one location, they did not cluster together, as can be seen for the seven samples obtained in Bouiblane (Bo), Figure 1. Likewise, pollen profile cluster analysis showed no correlation to the collection sites, Figure 2. Although part of this volatiles and pollen variability can be attributed to the specific local flora around each producer beehive, other factors, not evaluated in the present study, cannot be ruled out, such as specific climatic conditions in the harvesting year, the harvesting procedure, honey processing before bottling, such as heating and filtration, the storage temperature, and storage time, among others. For instance, due to honeýs composition, several reactions can occur at high temperatures, since carbohydrates and free amino acids are responsible for the generation of furan and pyran derivatives, in Maillard reactions. Strecker degradation reactions can also occur between amino acids and dicarbonyl

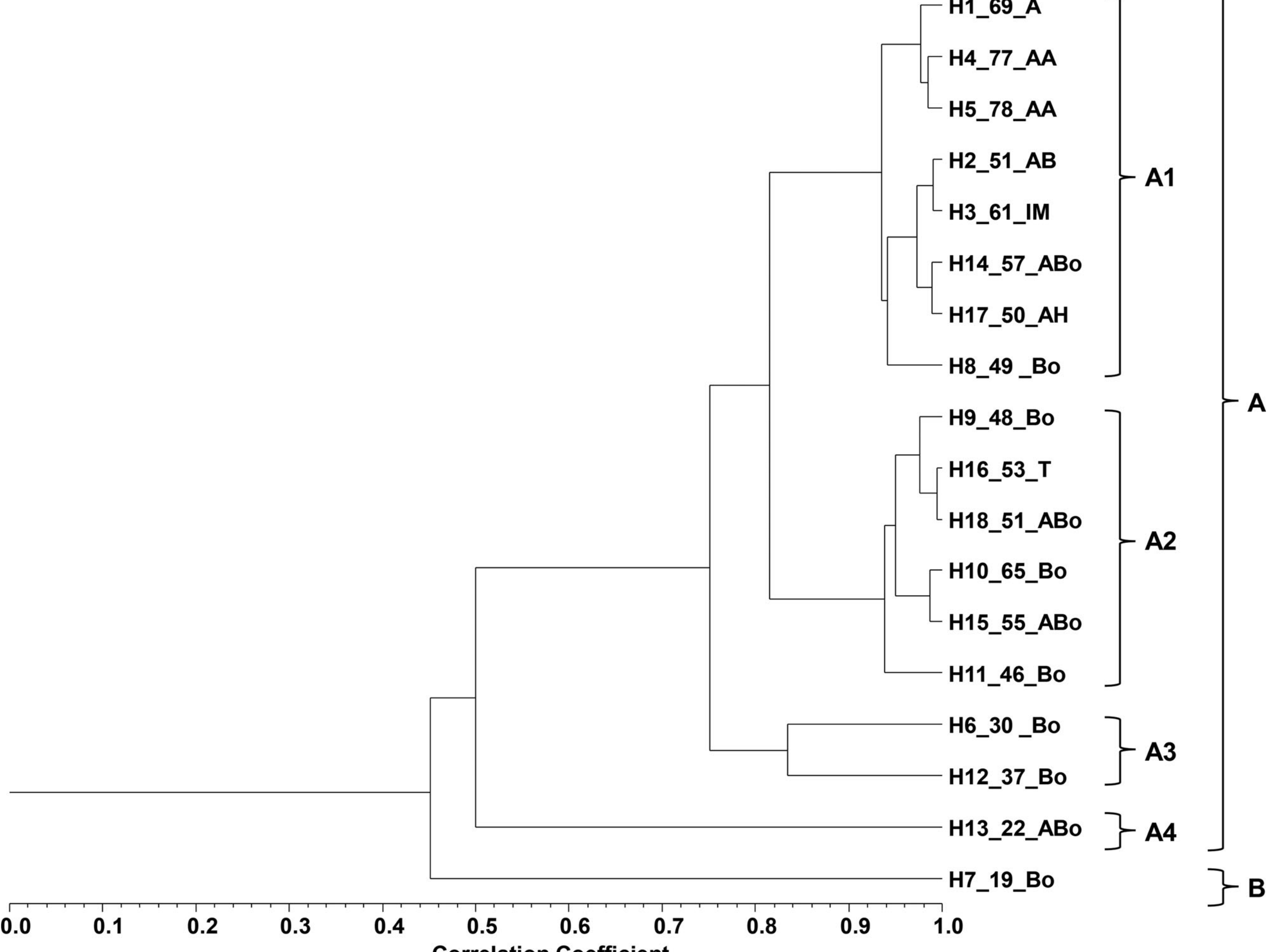

Figure 2. Dendrogram obtained by cluster analysis of *Bupleurum spinosum* honey percentage of pollen profiling, based on correlation and using unweighted pair-group method with arithmetic average (UPGMA). H: honey samples H1 to H18, with reference to *B. spinosum* pollen dominance after the underscore. For collection site codes, *vide* Table S1 (Supplementary File).

Table 2. Minimum and maximum percentage range composition of pollen profiling in *Bupleurum spinosum* honey samples. For samples grouped on each of the clusters and subclusters see Figure 2.

	Pollen profiling in *Bupleurum spinosum* honey							
	Cluster A							Cluster B
	Cluster A1		Cluster A2		Cluster A3		Cluster A4	
Plant Genus or Species*	Min	Max	Min	Max	Min	Max		
Bupleurum spinosum	49.2	78.2	45.9	64.5	30.0	37.1	21.8	19.0
Phoenix sp.						0.9		
Lactuca sp.						0.6		
Raphanus sp.		4.1				6.6		4.1
Celtis sp.		7.0				0.9		
Erica sp.		1.1						
Euphorbia sp.		7.4			5.9	14.7	1.9	
Cytisus sp.		9.9	22.9	38.3	2.8	8.8		45.8
Mimosa sp.		1.9						
Quercus sp.		5.1			2.3	2.5		
Salvia sp.				6.1	2.7	17.4	2.1	2.7
Artocarpus altilis	2.4	18.4		5.6		1.9	28.3	
Eucalyptus sp.		2.5		12.4		8.9		
Phytolacca americana		2.4		10.4	4.1	4.1		
Pinus sp.		3.6						
Eragrostis pilosa		19.9		11.9	3.0	5.8	26.1	3.3
Prunus sp.		10.9						
Citrus sp.				2.1		0.9		
Populus sp.	6.4	19.4	1.4	12.3	20.6	21.6	19.8	15.1
Unknown								10.0

*For each species family *vide* Table S1 (Supplementary File).

compounds producing aliphatic and aromatic aldehydes (Jerković et al., 2010; Silva et al., 2021).

In the present study with *Bupleurum* honey, 2-acetylfuran, benzaldehyde, 5-methyl furfural, and benzene acetaldehyde were identified in all honey samples, being described as volatile artifacts formed during honey heating and storage according to Jerković et al. (2019). These compounds are thus ubiquitous to several monofloral honeys and cannot be considered as reliable markers for monofloral honey types.

On the other hand, other compounds, also identified in all studied *Bupleurum* honey samples, such as ethyl oleate [traces (t)-49%], 1-phenyldodec-1-en-3-one (t-25%), and 6-methyl-6-(5-methylfuran-2-yl) heptan-2-one (t-9%), are less common, or unreported, in other monofloral honeys volatiles. To what extent these compounds can be considered as volatile markers for *Bupleurum* honey depends on further studies with a larger number of samples obtained from different geographical origins.

Although there was a fairly good correspondence between the groups defined by volatiles and pollen cluster analysis, the fact that the sample H3_61, with

the dominance of *B. spinosum* pollen was the only member of Cluster B in volatiles analysis and that, on the other hand, sample H7_19, with the lowest *B. spinosum* pollen content, was included in Cluster A in volatiles analysis, may indicate that in addition to *B. spinosum* pollen other species pollen contribute to the overall volatiles characteristics of this honey.

Moreover, the volatile compounds of honey depend also, if not mainly, on the flower volatiles, on the nectar, and/or honeydew collected by the bees. Despite the existence of studies on different *B. spinosum* aerial parts, extracted either with hexane (Barrero et al., 1998), supercritical CO_2 (Maxia et al., 2011), or as essential oils (Casiglia et al., 2016; Maxia et al., 2011; and references therein), no studies addressed this species' flower volatiles. Likewise, no studies have evaluated this species' nectar volatiles. These evaluations would be relevant to seek the presence of compounds that could be used as markers in the botanical characterization of this monofloral honey.

Floral odours contribute to plant-pollinator interaction since they are used by pollinators to locate pollen and nectar rewards (Burkle & Runyon, 2019). Therefore, flower volatile compounds have been suggested as the main contributors to visitation decisions by pollinators. The concentration of flower volatile compounds can vary due to genetic differences among subspecies and plant populations in different locations (Klatt et al., 2013), which may contribute to the differences found in honey volatiles with the same floral origin, although with varied geographical origins.

Honey contains pollen grains from different botanical species, and those from wind-pollinated or nectarless plants do not contribute to honeys´ botanical origin, being referred to as poliniferous plants pollen grains, Table S1 (Supplementary File). On the other hand, pollen grains from nectariferous plants determine honeýs botanical origin (Louveaux et al., 1978; Maia et al., 2005). Comparing the representativity of polleniferous plants in pollen profiling of *B. spinosum* honeys with volatiles cluster analysis, Table S1 (Supplementary File) and Figure 1, Cluster A2 showed the highest number of honeys, three out of six, with $\geq$10% pollen from each polleniferous species, from Poaceae and Salicaceae (H15_55, H14_57, and H13_22). Clusters A1 and A3 followed, and Cluster A4 evidenced the lowest percentage of pollen of polleniferous species. The only sample from Cluster B showed 13% and 10% of pollen from polleniferous species from Poaceae and Salicaceae, respectively.

The predominance of the pollen type in the honey varies according to the climate, being a factor that promotes variability to be considered in the botanical origin of honey, according to several authors that studied the influence of bioclimatic areas as well as floral and geographical origin in Apiaceae honeys from Algeria (*Foeniculum* sp., *Daucus* sp., *Coriandrum* sp., *Eryngium* sp., *Pimpinella* sp.) and Argentina (*Ammi majus*, *Eryngium* sp., *Mulinum spinosum*) (Aloisi et al., 2013; Homrani et al., 2020; Patrignani et al., 2018). This finding is in line with the present study, as different pollen types were identified in the samples from distinct regions, with different floral origins and thus contributing to the identified volatiles in the honey samples.

Despite being considered a low contributor to honey aroma, pollen of several plant species has distinctive fragrances as evaluated by the human nose and confirmed by GC and GC–MS. Some of the volatile compounds may have evolved as a defence against pollen-feeding animals, although when plants became dependent on animals for pollination, they may have also assumed an attractive role. Floral scents studies are important to better understand the chemical bases of plant-animal relationships as well pollination ecology (Dobson & Bergström, 2000; Flamini et al., 2007).

Broader studies of pollen volatiles from species whose pollen occurs in the honey samples would allow determining the presence of putative species-specific volatile marker compounds and complement the global view on the importance of pollen profile on Zantaz honey volatiles.

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Disclosure statement

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