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Additional Information

Palynological and chemical volatile components of typically autumnal honeys of the western Mediterranean

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Abstract

Twenty-five samples of autumnal honeys from the western Mediterranean (Mallorca and Eivissa, Balearic Islands) were examined for pollen content (qualitative and quantitative melissopalynological analysis), moisture, electrical conductivity, colour, sensorial qualities and volatile components. Quantitative analysis showed that the honey contained Maurizio's Class II: 64%, Class III: 28%, Class IV: 4% and Class V: 4%. Fifty-four pollen types, with an average number of 16.68 per sample, were identified, belonging to 29 botanical families. Only two taxa (Ceratonia siliqua and Erica multiflora) were found in all samples. Seventeen samples were unifloral (68%) – ten (40%) of C. siliqua, six (24%) of E. multiflora and one (4%) of Hedera helix. All honeys have a low honeydew index (<0.09%), while the values for electrical conductivity and water content were high. The major honey volatile components are: cis- and trans-linalool oxides (64.2%) and hotrienol (10.4%) for the carob (C. siliqua) andtrans-linalool oxide (13.4%), p-menthane-1,8-diol (11.1%), safranal (9.7%), limonene (5,4%), α-pinene (3.7%) and oxoisophorone (3.4%) for the winter heather (E. multiflora).

Keywords: carob (Ceratonia siliqua), winter heather (Erica multiflora), ivy(Hedera helix), Balearic Islands, volatile compounds

Introduction

In a Mediterranean context, the human colonisation of the Balearic archipelago took place quite late. Archaeological data relating to the first stable settlement suggest human presence in the area between 4400 and 3600 BP (Calvo et al., 2002). This region has a long history of beekeeping extending back at least 2500 years (Bonet & Mata, 1997) and includes structures such as the stone beehives of the Punic, Greek and Roman civilisations on the island of Eivissa. In the abundant production of autumnal honeys, traditionally, beekeepers recognised that Apis mellifera L. visits flowers of Ceratonia siliqua L., Erica multiflora L., Diplotaxis erucoides (L.) DC., Dittrichia viscosa (L.) Greuter, Hedera helix L. and Rosmarinus officinalis L. These are the predominant autumn flowering species, but there are also a several others (e.g. Herrera, 1986, 1988; Guardia et al., 1998; Pons, 2002). One aspect of honey production is the intensive cultivation of carob trees(Ceratoniasiliqua).Ceratonia siliqua honey is limited to warmer Mediterranean territories (Balearic Islands, Sicily, Sardinia, Greece, Turkey, northern Africa and France), where it has been mostly studied (Sáenz Lain and Gómez Ferreras (2000))reported that in Spain, this honey type is only present in the Balearic Islands, but there is no reference to any physico-chemical parameters. However, Ceratonia pollen appears in some honeys from Israel, Morocco, Algeria and the southern Iberian Peninsula (Damblon, 1988; Ricciardelli d'Albore, 1998; Persano Oddo et al., 2000; Ricciardelli d'Albore & Intoppa, 2000; Terrab et al., 2000, 2001, 2003a).

Figure 1. Map of Balearic Islands (upper part) and map with distribution of the apiaries investigated (lower part). Collection sites: 1, 9, 11 – Binissalem; 2, 6, 7, 8, 14 – Campanet; 3 – Puigpunyent; 4 – Llubí; 5, 25 – Petra; 10 – Biniamar; 12 – Pollença; 13 – Esporles; 15, 17 – Soller; 16 – Andratx; 18, 19, 20 – Artá; 21 – Estellencs; 22, 23 – Eivissa; 24 – Orient.

Erica multiflora is a common species in dry and calcareous coastal shrubland regions, and its unifloral honey is common in the eastern Iberian Peninsula, Malta and Croatia; it is found occasionally in Italy and Tunisia (Persano Oddo & Piro, 2004). Erica manipuliflora Salisb., a similar species, found in the phryganas or garrigue of the central and eastern Mediterranean region, can also be unifloral. Other unifloral Erica honeys have been studied in northern Spain and Portugal, but belong to other species such as E. umbellata L., E. australis L. and E. arborea L. (Sà-Otero et al., 1991, 2006; Seijo et al., 1992; Sáenz Lain & Gómez Ferreras, 2000) or Calluna sp. (Andrade et al., 1999). Hedera helix honey is rarely found in Spain, Italy, France or Croatia (Persano Oddo & Piro, 2004). One of the main features of the Mediterranean climate is the dry summers (Rivas-Martínez, 1995), with concentrated rainfall during winter or spring. Some territories in the western Mediterranean, however, have their maximum rainfall during the autumn (Llasat et al., 1996). This pattern of autumnal rain is located in the thermo-Mediterranean belt (Rivas-Martínez, 1983), and it favours an abundance of autumnal-flowering plants. The Balearic Islands, located in the centre of the western Mediterranean region, represent an area that has an autumn locally known as 'winter spring'. Honey aroma has been studied for years in order to develop analytical methods for its determination based on the volatile organic fraction. The composition of the honey's volatile fractions derives from its floral origin and from the foraging habits and physiology of the bees. For this reason, the chemical volatile composition is of great importance in characterising the floral and/or

geographical origin of honeys. Volatile components have been determined as specific markers for unifloral honeys. The use of dynamic heat space techniques (Bouseta et al., 1992; Radovic et al., 2001) or solid-phase micro extraction could prove to be useful for testing the authenticity of the botanical origin. Marker components of unifloral honeys can probably be detected among the less volatile components (e.g. Guidotti & Vitali, 1998; Pérez et al., 2002; Wolski et al., 2006). In different honeys, a range of natural organic substances of the plant source have been identified as phytochemicals(e.g.de Maria&Moreira,2003;Castro-Vázquez et al., 2009) and several markers are identified for the determination of the floral origin of the respective honeys (e.g. Radovic et al., 2001; Piasenzotto et al., 2003; Cajka et al., 2009; Melliou & Chinou, 2011). The main objectives of this study of autumn/ winter honeys from the western Mediterranean region are: (a) melissopalynological characterisation;

Figure 1. Map of Balearic Islands (upper part) and map with distribution of the apiaries investigated (lower part). Collection sites: 1, 9, 11 – Binissalem; 2, 6, 7, 8, 14 – Campanet; 3 – Puigpunyent; 4 – Llubí; 5, 25 – Petra; 10 – Biniamar; 12 – Pollença; 13 – Esporles; 15, 17 – Soller; 16 – Andratx; 18, 19, 20 – Artá; 21 – Estellencs; 22, 23 – Eivissa; 24 – Orient.

(b) evaluation of the physicochemical parameters (colour, humidity, conductivity) and organoleptic characteristics; (c) characterisation of the major volatile components of the more specific honeys; (d) the establishment of markers that can help to distinguish these honeys from those with a different geographical origin.

Material and methods

Study area This study analysed 25 honey samples collected in autumn/winter from Mallorca (2004/2005) and Eivissa (2006/2007) (Figure 1). The climate of the sampling localities corresponds to the Mediterranean maritime type (de Bolòs, 1985) of mild winters, with a maximum rainfall in autumn and at the end of winter or beginning of spring (de Bolòs & Vigo, 1984–2001). According to the bioclimatic classification of Rivas-Martínez (1995, 1997), the study area has a Mediterranean macrobioclimate, a Mediterranean pluviseasonal-oceanic or xericoceanic bioclimate and a thermo-Mediterranean thermoclimate. Certain zones in the study area have a diverse ombroclimate. In general, the semi-arid, dry and sub-humid ombrotypes are common, whereas in southern Mallorca and in Eivissa the dry type prevails. Vegetation around the beehives consists of a mix of carob culture (different varieties, mainly Bugadera, De la mel, Vera, Costella and Duraió in Mallorca, and Panesca, Boval and Uraiona in Eivissa), scrub communities and, in some localities, rupicolous vegetation.

Beekeeping For beekeeping, the standard Langstroth beehive is generally used. The extracted honey is produced by centrifugation. The honeybee species Apis mellifera is identified as the Western-European race (de la Rua et al., 2002).

Melisso palynological analysis in order not to damage the pollen or to destroy fungal spores, the qualitative analysis of the samples was prepared without acetolysis (Louveaux et al., 1978; Terrab et al., 2004; von der Ohe et al., 2004). Qualitative analysis of melissopalynological characters were carried out by a standardised method of Louveaux et al. (1978). While it has been demonstrated that water dilution results in lower yields that can be biased against certain taxa (Jones & Bryant, 2004) due to the crystallised nature of some samples, we used

warm water dilution to aid dissolution ofthecrystallisedhoney.Thesampleof10gofhoney was dissolved in 20ml of distilled water at 30∘C for 10min with continuous stirring. Two 10ml subsamples were centrifuged two times for 10min at 2200g (at 3500rpm; cf. Pendleton, 2006). The sediment was centrifuged and dehydrated with different ethanol concentrations (50%, 70% and 96%). The pure sediment was stained with glycerine-fuchsine, mounted on microscope slides and examined under an Olympus BX41 microscope. Two samples were analysed and >700 pollen grains were identified and counted with an Olympus BX41 microscope at 400× or 1000× magnification. According to the qualitative analysis, the pollen types were identified on the lowest possible taxonomic level (family, genus, species or type). For each pollen type, in relation to the total number of pollen grains recognised, the frequency and percentage were calculated. The pollen types identified and counts were classified into five different categories (Louveaux et al., 1978) according to percentage of occurrence: dominant pollen (D, >45% of the total pollen grains counted); secondary pollen (S, 16–45%); important minor pollen (I, 3–15%); minor (trace) pollen (m, <3%); and sporadic pollen (p, <1%). For the quantitative analysis, natural honey (Vorwohl, 1967) was used following the methodology proposed by La Serna et al. (1999) and La Serna and Gómez (2006). The pollen grains were counted from a homogenised suspension of 10g honey in 20ml of distilled water, using a haemocytometer (Fuchs–Rosenthal counting chamber) and an optical microscope at 40× magnification. The result is expressed as the number of pollen grains per gram of honey and the samples have been assigned Maurizio's classes (Maurizio, 1939, 1979): I, <2000 grains; II, 2000–10000; III, 10000–50000; IV, 50000–100000; V, >100000. According to the criteria of Louveaux et al. (1978) and Maurizio (1979), a honey is considered unifloral if the pollen frequency of one plant is larger than 45% with exceptions to this due to 'under' and 'over' representation. The honeydew index (HDE/P) was calculated from the ratio of honeydew elements and pollengrains ofnectariferous plants (Louveaux etal., 1978).

Physicochemical parameters Determination of the physicochemical parameters such as colour, electrical conductivity and water content may help with the identification of certain honey types (Bogdanov & Martin, 2002). Colour was measured in mm Pfund. Values in the range 84–114 mm were considered as amber, over 114 mm as dark or very dark. Determinations were assessed with a digital Hanna C-221 colorimeter (Hanna Instruments Ltd, Leighton Buzzard, UK). The electrical conductivity of a 20% honey solution in distilled water was measured at 20∘C with a Hanna Dist3 conductimeter (Hanna Instruments Ltd) and the values were expressed as µS/cm. Water content (moisture) was determined with an Atago HHR-2N refractometer (Atago Co. Ltd, Tokyo, Japan) reading at 20∘C, using the Wedmore table (AOAC, 1990). This evaluation was important in order to verify the authenticity of unifloral honey, since it can reveal the presence of botanical components not detected by melissopalynological and physico-chemical analysis. For the sensorial analysis of honey (visual, olfactory and gustative sensations), the method of Piana et al. (2004) was used.

Table I. Pollenty pes and frequency of each type found in the samples as dominant (D, >45%), secondary (S, 16–44%), important pollen (I, 3–15%), minor pollen (m, 1–3%) and pollen present (pp, <1%).

Table I. Pollen types and frequency of each type found in the samples as dominant (D, >45%), secondary (S, 16–44%), important pollen (I, 3–15%), minor pollen (m, 1–3%) and pollen present (pp, <1%).

Pollen taxa	D	S	I	m	pp	BV
Apiaceae						
Foeniculum vulgare	—	—	—	1	11	N, P
Araliaceae						
Hedera helix	1	1	7	3	7	N, P
Arecaceae						
Phoenix spp.	—	2	11	3	5	N, P
Asteraceae						
Bellium spp.	—	—	—	—	2	N, P
Calendula arvensis	—	—	1	1	5	N, P
Cirsium / Carduus	—	—	1	2	9	N, P
Dittrichia viscosa	—	1	6	5	7	N, P
Erygeron spp.	—	—	—	1	—	
Senecio spp.	—	—	—	—	1	N, P
Sonchus spp.	—	—	—	1	4	N, P
Boraginaceae						
Echium spp.	—	—	1	—	2	N, P
Brassicaceae						
Biscutella type	—	—	1	1	1	N, P
Brassica type	—	—	1	1	4	N, P
Diplotaxis erucoides	1	1	8	5	7	N, P
Caesalpinaceae						
Ceratonia siliqua	11	8	6	—	—	N, P
Caryophyllaceae						
Silene spp.	—	—	—	—	2	N, P
Chenopodiaceae/Amaranthaceae	—	—	—	—	6	P
Cistaceae						
Cistus spp.	—	—	4	4	13	P
Helianthemum spp.	—	—	1	—	1	P
Cupressaceae						
Cupressus / Juniperus	—	—	—	1	1	P
Ericaceae						
Arbutus unedo	—	—	2	—	6	N, P
Erica multiflora	7	6	10	1	1	N, P
Euphorbiaceae						
Euphorbia spp.	—	—	1	—	2	N, P
Fabaceae						
Acacia spp.	—	—	—	—	1	N, P
Anthyllis cytisoides	—	—	1	—	3	N, P
Astragalus spp.	—	—	1	1	—	N, P
Dorycnium pentaphyllum	—	—	—	—	6	N, P
Genista spp.	—	—	—	3	5	N, P
Lotus spp.	—	—	—	4	4	N, P
Medicago spp.	—	—	2	3	5	N, P
Trifolium spp.	—	—	1	1	3	N, P
Vicia spp.	—	—	—	1	1	N, P
Fagaceae						
Quercus ilex	—	—	—	1	3	P
Lamiaceae						
Lavandula spp.	—	—	—	1	5	N, P
Rosmarinus officinalis	—	—	3	—	6	N, P
Teucrium spp.	—	—	1	1	2	N, P
Micromeria inodora	—	—	2	—	—	N, P
Thymbra capitata	—	—	—	—	1	N, P
Liliaceae						
Asparagus spp.	—	—	8	5	5	N, P
Asphodelus ramosus	—	—	—	—	3	N, P
Myrtaceae						
Myrtus communis	—	1	2	2	1	N, P
Eucalyptus spp.	—	—	3	2	8	N, P

(*Continued*)

Table I. (*Continued*).

Pollen taxa	D	S	I	m	pp	BV
Oleaceae						
Olea europaea	—	—	1	—	4	P
Oxalidaceae						
Oxalis pes-caprae	—	—	—	1	3	P
Pinaceae						
Pinus halepensis	—	—	—	—	1	P
Plantaginaceae						
Plantago spp.	—	—	—	—	6	P
Poaceae	—	—	—	—	7	P
Ranunculaceae						
Clematis cirrhosa	—	—	2	—	1	P
Rosaceae						
Eriobotrya japonica	—	—	2	3	12	N, P
Rubus ulmifolius	—	—	—	1	1	N, P
Rhamnaceae						
Rhamnus alaternus	—	—	—	3	7	N, P
Rutaceae						
Citrus aurantium	—	—	—	2	8	N, P
Scrophulariaceae	—	—	—	2	2	N, P
Smilacaceae						
Smilax aspera	—	—	—	2	6	P

Note: Bold, family names; BV, beekeeping value; N, nectariferous species; P, polliniferous species.

Chemical analysis

The samples of the more characteristic unifloral honeys of Ceratonia siliqua and Erica multiflora were analysed for volatile organics. Extraction of the volatile organic fraction followed the methodology of Verzera et al. (2001). Solid phase microextraction (SPME) was used to extract the organic volatile components of the honey without adding solvents. The SPME fibre used was covered with polydimethylsiloxane/divinylbenzene (PDMS/DVB) with a 65μm film thickness. A honey sample of 16g was dissolved with 2g sodium chloride (NaCl) in 7ml water in a 40ml glass headspace vial. The vial was gently heated to 30◦C and stirred for 30min. Subsequently, the SPME fibre was exposed to the vapour phase with the sample for 25min with stirring. Each sampling of the differentvarieties of honey was made in triplicate. Samples were then analysed using a gas chromatograph-mass spectrometer (GC-MS). The SPME fibre was introduced onto the GC injector in splitless mode and held for 3 min to permit the complete thermic (250◦C) secretion of the compounds from the fibre onto the column. The extracted volatiles enter into the system of gas chromatography-mass coupled to spectrometry-mass (GC-EM) for detection and identification. A Clarus 600 GC-MS (Perkin–Elmer, Inc., Wellesley, MA, USA) with a capillary column ZB-5MS (30m length×0.25mm internal diameter×0.25mm film thickness; Phenomenex, Inc., Torrance, CA, USA) was used to analyse the samples.

Figure 2. Distribution of the samples in frequency classes according to their richness in pollen types; X: number of the pollen, Y: percentage of samples.

The injector temperature was 220◦C. The temperature program followed this schedule: 60◦C for 5min, increasing to 180◦C at rate of 3 ◦C/min, thereafter by 20◦C/min until 280◦C, holding this temperature for 10min. The carrier gas was helium at a constant flux of 1ml/min. Detection used an electron impact (EI) with energy (ionisation potential) of 70eV, while the ionisation source up to 180Â◦C. Mass spectra were obtained in scan mode in a mass range of 45–300uma. The chromatograms and spectra of the samples were processed using the GC-EM software, Turbomass version 5.1 (Perkin-Elmer, Inc.). A mixture of n-alkanes (C8–C40)

dissolved in n-hexane was supplied by Supelco (Bellefonte, PA, USA) to retain index determinations. The control was done between n-octane and n-eicosane. For components with retention indices (RI) less than 800, extrapolation using n-octane and n-nonane was employed. Main volatile honey components were identified taking the RI (determined with reference to a homologous series of normal alkanes) into account, and by comparing their mass spectral fragmentation patterns (Adams, 2007) and stored on the MS library [NIST MS Search Program (version 2.0)]. For the quantitative component, the peak areas were considered according to the concentrations from GC.

Results Melissopalynological analysis Qualitative analysis yielded a total of 54 pollen types (Tables I–III) belonging to 29 botanical families. Most of the samples were low in pollen types with a mean value of 16.7±5.21 (Figure 2). Only two taxa (Ceratonia siliqua and Erica multiflora) were present in all samples (Table I), five taxa (Hedera helix, Phoenix sp., Dittrichia viscosa, Diplotaxis erucoides and Cistus sp.) were found in more than 75% of samples and three taxa [Eryobotria japonica (Thunb.) Lindl.,
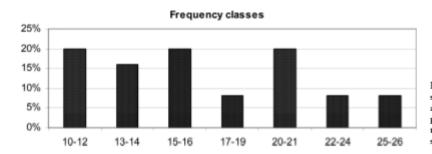

Figure 2. Distribution of the samples in frequency classes according to their richness in pollen types; *X*: number of the pollen, *Y*: percentage of samples.

Asparagus acutifolius L. and Myrtaceae] were present in 50–75% of the samples. According the Maurizio's classification of the quantitative analysis, 64% of the samples belonged to Class II, 28% to Class III and 8% to Class IV and V, respectively. The overall pollen content can be considered as medium (pollen density 4438– 135500grains/g, average 18484grains/g; Table II). The nectariferous taxa represent 78% of the taxa, while polliniferous taxa are represented by 3%. The honeydew index is very low in all samples (below 0.09), thus can be regarded as practically nil (Loveaux et al., 1978) and is here ignored.

Physicochemical parameters

The dominant colour in 22 samples was dark, amber colour was found in three samples only. Most of the samples have high or very high values for electrical conductivity (410–1780 µS/cm). The water content varied between 16.1% and 22% (Table II).

Honey types Seventeen honeys were unifloral (ten samples of Ceratonia siliqua, six of Erica multiflora and one of Hedera helix), and eight were multifloral (Tables II, III). In the Ceratonia siliqua honey, between 11 and 22 types of pollen were found (average 18), with only one sample revealing an absence of Erica multiflora pollen (the second most common nectariferous species). Quantitative analysis revealed a mediumhigh pollen content (80% in Class II, 10% in Class IV and 10% in Class V; Table II). The pollen density ranged between 5292 and 135500 grains/g (average 28957). The physical state of this honey is liquid. The rate of crystallisation depends on the water content, but it is always very low (>1 year); however,

eventually, fine, powdery crystals are produced. When C. siliqua pollen predominates no or very low crystallisation occurs. The crystallisation process is linked to the presence of Erica nectar, which, when

Table II. Melissopalynological analysis and physicochemical parameters and frequency-class distribution.

Table II. Melissopalynological analysis and physicochemical parameters and frequency-class distribution..

Sample			Qualitative analysis	Quantitative analysis	Physico-chemical parameters		
N	UTM	Locality	Honey type	Maurizio's classes	Moisture (%)	Electrical conductivity (mS/cm)	Colour (mm PFUND)
1	31SDD89	Binissalem	**Multifloral** – *Erica multiflora* (29%) with *Ceratonia siliqua* (27%), *Diplotaxis erucoides* (43%) and *Hedera helix* (2%)	II	19.0	0.86	135
2	31SDD90	Campanet	**Unifloral** – *Ceratonia siliqua* (59%) with *Erica multiflora* (6%), *Diplotaxis erucoides* (25%) and *Dittrichia viscosa* (5%)	II	18.1	0.62	125
3	31SDD81	Puigpunyent	**Unifloral** – *Erica multiflora* (56%) with *Ceratonia siliqua* (25%), *Arbutus unedo* (6%) and *Hedera helix* (14%)	II	18.2	0.7	130
4	31SED09	Llubi	**Multifloral** – *Ceratonia siliqua* (41%) with *Phoenix* sp. (9%), *Diplotaxis erucoides* (6%) and *Dittrichia viscosa* (4%)	IV	18.1	1.36	150

N	UTM	Locality	Honey type	Maurizio's classes	Moisture (%)	conductivity (mS/cm)	Colour (mm PFUND)
1	31SDD89	Binissalem	**Multifloral** - *Erica multiflora* (29%) with *Ceratonia siliqua* (27%), *Diplotaxis erucoides* (43%) and *Hedera helix* (2%)	II	19.0	0.86	135
2	31SDD90	Campanet	**Unifloral** - *Ceratonia siliqua* (59%) with *Erica multiflora* (6%), *Diplotaxis erucoides* (25%) and *Dittrichia viscosa* (5%)	II	18.1	0.62	125
3	31SDD81	Puigpunyent	**Unifloral** - *Erica multiflora* (56%) with *Ceratonia siliqua* (25%), *Arbutus unedo* (6%) and *Hedera helix* (14%)	II	18.2	0.7	130
4	31SED09	Llubí	**Multifloral** - *Ceratonia siliqua* (41%) with *Phoenix* sp. (9%), *Diplotaxis erucoides* (6%) and *Dittrichia viscosa* (4%)	IV	18.1	1.36	150
5	31SED08	Petra	**Unifloral** - *Ceratonia siliqua* (51%) with *Dittrichia viscosa* (31%) and *Diplotaxis erucoides* (14%)	II	19.2	1.4	138
6	31SDD90	Campanet	**Unifloral** - *Ceratonia siliqua* (54%) with *Phoenix* sp. (7%), *Erica multiflora* (6%) and *Diplotaxis erucoides* (4%)	II	16.1	1.47	97
7	31SDD90	Campanet	**Unifloral** - *Ceratonia siliqua* (66%) with *Erica multiflora* (12%), *Phoenix* sp. (3%) and *Dittrichia viscosa* (3%)	II	16.6	1.71	106
8	31SDD90	Campanet	**Unifloral** - *Ceratonia siliqua* (79%) with *Erica multiflora* (10%), *Dittrichia viscosa* (5%) and *Phoenix* sp. (4%)	II	18.8	1.68	150
9	31SDD89	Binissalem	**Unifloral** - *Ceratonia siliqua* (47%) with *Erica multiflora* (28%), *Hedera helix* (14%) and *Diplotaxis erucoides* (4%)	III	16.4	1.78	150
10	31SDD89	Biniamar	**Multifloral** - *Ceratonia siliqua* (14%) with *Phoenix* sp. (16%), *Erica multiflora* (9%) and *Calendula arvensis* (3%)	II	17.7	1.17	106
11	31SDD89	Binissalem	**Multifloral** - *Ceratonia siliqua* (31%) with *Erica multiflora* (12%), *Diplotaxis erucoides* (10%) and *Dittrichia viscosa* (8%)	II	17.8	1.56	150
12	31SEE01	Pollença	**Unifloral** - *Ceratonia siliqua* (65%) with *Erica multiflora* (9%), *Dittrichia viscosa* (7%) and *Phoenix* sp. (2%)	II	17.4	1.69	150
13	31SDD86	Esporles	**Unifloral** - *Ceratonia siliqua* (45%) with *Erica multiflora* (14%), *Hedera helix* (8%) and *Phoenix* sp. (6%)	II	16.9	1.11	101
14	31SDD90	Campanet	**Unifloral** - *Ceratonia siliqua* (55%) with *Erica multiflora* (29%), *Phoenix* sp. (6%) and *Diplotaxis erucoides* (3%)	III	18.2	1.54	132
15	31SDE70	Soller	**Unifloral** - *Erica multiflora* (53%) with *Ceratonia siliqua* (35%) and *Hedera helix* (3%)	V	17.8	0.93	129
16	31SDD86	Andratx	**Unifloral** - *Ceratonia siliqua* (48%) with *Erica multiflora* (45%)	II	22.0	0.66	150
17	31SDE70	Soller	**Multifloral** - *Erica multiflora* (37%) with *Hedera helix* (21%), *Rosmarinus officinalis* (10%) and *Ceratonia siliqua* (6%)	III	17.8	0.63	130
18	31SED39	Artà	**Multifloral** - *Erica multiflora* (38%) with *Phoenix* sp. (33%) and *Ceratonia siliqua* (13%)	III	18.8	0.63	150
19	31SED39	Artà	**Unifloral** - *Erica multiflora* (56%) with *Ceratonia siliqua* (3%), *Phoenix* spp. (4%) and *Hedera helix* (3%)	III	20.1	0.59	150
20	31SED39	Artà	**Unifloral** - *Erica multiflora* (70%) with *Ceratonia siliqua* (11%), *Phoenix* spp. (3%) and *Hedera helix* (2%)	III	17.4	0.41	150
21	31SDD59	Estellencs	**Unifloral** - *Erica multiflora* (54%) with *Ceratonia siliqua* (26%), *Arbutus unedo* (6%) and *Hedera helix* (4%)	II	18.5	0.99	150
22	31SCD51	Eivissa	**Unifloral** - *Erica multiflora* (62%) with *Ceratonia siliqua* (19%), *Rosmarinus officinalis* (4%) and *Micromeria inodora* (3%)	II	19.0	0.8	140
23	31SCD72	Eivissa	**Multifloral** - *Ceratonia siliqua* (42%) with *Erica multiflora* (40%), *Rosmarinus officinalis* (5%) and *Micromeria inodora* (3%)	II	16.8	0.58	134
24	31SDD79	Orient	**Unifloral** - *Hedera helix* (46%) with *Erica multiflora* (14%), *Ceratonia siliqua* (8%) and *Phoenix* sp. (4%)	II	18.0	1.26	150
25	31SED08	Petra	**Multifloral** - *Ceratonia siliqua* (28%) with *Diplotaxis erucoides* (31%) and *Erica multiflora* (6%)	III	18.6	0.72	110

Note: N, the sample number; UTM, coordinates of locality samples.

25 31SED08 Petra Multifloral - Ceratonia siliqua (28%) with Diplotaxis erucoides (31%) and Erica multiflora (6%) present, causes the formation of fine crystals with a powdery appearance. When the honey is liquid, its colour is dark or amber, but when it is crystallised, it is lighter. The odour is intense and has smells of flowers, liquorice, wood, mushrooms and forest. The taste is sweet, a little salty with a touch of bitterness, caramel, liquorice, humus, mushrooms and forest. A retronasal aroma that is somewhat floral, wood, forest, liquorice, which clears the nose. In the Erica multiflora honey, between 11 and 16 pollen types occur (average 15.58), with 40% of the samples being characterised by the presence of up to 6% Arbutus unedo L. pollen (an underrepresented type that adds a very singular flavour). Only 20% of the samples

contained Ceratonia siliqua pollen. Quantitative analysis (Table II) showed a medium pollen content (50% Class II, 50% Class III). The pollen density ranges between 4438 and 23547 grains/g (average 12030). The organoleptic analysis shows the physical state can transition from liquid to crystal rapidly (crystals may appear in one month). The crystals are fine, similar to powder or grit, and sometimes, there may appear separated phases. The colour when liquid is dark (toffee or dark brown) with an orange tonality, but when it is crystallised, there is a remarkable orange colour variation. The taste is sweet, somewhat salty, acidic, humus, mushrooms and forest with a final bitter taste. The odour suggests caramelised sugar and is sometimes strong, intense, fresh and flowery. A retronasal aroma is of forest and syrup. Due to the harvest period, these honey types may have high moisture content. This fact and their rapid rate of crystallisation create problems for its preservation. The only sample analysed of Hedera helix honey had a pollen density of 7828grains/g (Class II) and 18 pollen types (predominantly from nectariferous species; Table II). The organoleptic analysis reveals that the physical state is extremely smooth. When crystallisation occurs, it is very fine, which is the reason for the honey's smooth texture. These characteristics will remain for about two years. The colour is dark (brown leather, toffee dark brown) and with an odour of liquorice and forest that is intense. The taste is acidic, bitter, medicines, roasted and coffee. A retronasal aroma of liquorice expectorant was noted. Multifloral honey contains between ten and 25 types of pollen per sample (average 18.5). This type of honey contains different proportions of Ceratonia siliqua and Erica multiflora (Classes II or III) and the pollen density range from 5786 to 16797grains/g (average 11564; Table II). The organoleptic analysis reveals that the physical state has fine crystals. The colour varies from amber to dark. The odour is intense with smells of liquorice, forest, wood, caramelised sugar and flowery. The taste is sweet, salty, caramel and liquorice, humus, mushrooms and forest. A retronasal floral aroma with wood, forest, liquorice and syrup can be detected. This type of honey has different fractions of C. siliqua and E. multiflora, but the flavour and other characteristics of the C. siliqua predominate.

Chemical volatile analysis

The main volatile components of Ceratonia siliqua and Erica multiflora samples showed significant differences depending on the floral source of the honey. For example, the C. siliqua honey has a high content of linalool derivate, such as translinalool oxide (57.7%), cis-linalool oxide (6.6%) and hotrienol (10.4%). However, in Erica multiflora honey, components such as cis-linalool oxide, linalool and hotrienol are absent and trans-linalool oxide is present in lower levels. This type of honey is characterised by the presence of p-menthane-1,8diol (11.1%), safranal (9.7%), limonene (5.4%) and oxoisophorone (3.4%) and significant amounts of α-pinene and camphene (Table IV).

Discussion

Melissopalynology is recognised as the most applicable method in order to determine the botanical and, in many cases, geographical origin of a honey sample (Anklam, 1998). The pollen spectrum of the honey usually reflects the vegetation of the area, where the beehives are located (La Serna & Gómez, 2006). In some places of the south-western Mediterranean region, autumn, the rainiest season, represents a period characterised by intense flowering of a limited number of species (Kummerov, 1983; Arroyo, 1988; Thompson, 2005). The identified

pollen reflects the current natural vegetation of the two islands under study. The common flowering species at this time of the year are Ceratonia siliqua, Diplotaxis erucoides (from disturbed areas), Dittrichia viscosa, Phoenix sp. (cultivated), Hedera helix (wild and cultivated), Erica multiflora and Rosmarinus officinalis (abundant in the scrublands), which could be of a more general interest. Species such as Arbutus unedo and Micromeria inodora (Desf.)Benth.,with a more limited wild distribution, could have a more specific ecological or biogeographical importance (de Bolòs & Molinier, 1984; Llorens et al., 2007). Palynological investigation reveals that the autumnal honeys of Mallorca and Eivissa have no remarkable differences in their pollen spectra and shows a medium presence of pollen types, with clear dominance of a few species, such as Ceratonia siliqua, Erica multiflora and, locally, Rosmarinus officinalis. The presence of Micromeria inodora in the honeys of Eivissa is remarkable (sample 22: unifloral E. multiflora and sample 23: multifloral; Table III). This species can be considered a territorial trace guide species and a strong geographical marker for honeys produced in the Pityusic Islands (Eivissa and Formentera). Besides the importance of C. siliqua and E. multiflora, it is also evident that 72% of these autumnal honeys are unifloral. Most honeys have a high value for electrical conductivity, which is related to their mineral content according to their botanical origin (Vorwohl, 1964). In the autumnal honeys, this fact seems to be determined by the presence of Ceratonia siliqua honeydew (as fruit exudates). However, quantitative analysis shows that most honeys do not contain HDE and, according to Louveaux et al. (1978), this character indicates that nectar is the main source of the autumnal honeys. The water content of honey reflects the relation between the climatic conditions and the season of production (Conti et al., 2007; Mohammed & Babiker, 2009) and consequently, the autumnal honeys may have a high water content value. Moreover, these values are similar to the results measured in other autumnal honeys (c. 21%) such as Arbutus unedo (Persano Oddo et al., 2004) or various Ericaceae (Crane, 1975, 1990). According to the ranges established by the European Community Directive, this value can be considered as high (The Council of the European Union, 2002) and finally this does not reduce the quality of the honey.

Table III. Results of the qualitative analysis, represented as percentages.

Taxa/Sample number	2	5	6	7	8	9	12	13	14	16	24	3	15	19	20	21	22	1	25	4	10	11	17
Araliaceae																							
Hedera helix	1	<1	–	–	–	14	<1	8	<1	<1	46	4	3	3	2	4	–	2	–	1	1	<1	21
Asteraceae																							
Bellium spp.	<1	–	–	–	–	–	–	–	–	–	–	–	–	–	<1	–	–	–	<1	–	–	–	–
Calendula arvensis	–	–	3	–	–	–	–	–	1	1	–	–	–	–	<1	<1	–	–	<1	–	3	–	–
Cirsium/Carduus	–	–	2	–	–	–	1	1	<1	–	<1	–	<1	–	<1	–	–	–	–	1	<1	<1	<1
Erigeron spp.	5	31	1	3	5	<1	7	1	1	–	<1	<1	–	<1	2	–	1	<1	–	4	<1	8	–
Dittrichia viscosa	–	<1	<1	–	–	–	–	–	–	–	–	–	–	–	<1	–	1	–	–	<1	–	–	–
Senecio spp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–
Sonchus spp.	–	–	<1	–	–	–	<1	–	–	–	–	–	–	–	–	–	–	<1	<1	<1	–	–	–
Boraginaceae																							
Echium type	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Brassicaceae																							
Brassica type	–	–	–	–	–	–	3	–	–	–	1	–	–	–	–	–	–	<1	<1	–	<1	–	<1
Biscutella type	–	–	4	1	<1	4	<1	–	3	<1	2	<1	<1	1	<1	<1	5	43	7	6	2	3	–
Diplotaxis erucoides	25	14																	26			10	–
Caesalpinaceae																							
Ceratonia siliqua	59	51	54	66	79	47	65	45	55	48	8	25	35	5	11	26	19	27	28	41	14	31	6
Caryophyllaceae																							
Silene spp.	–	–	<1	<1	<1	–	<1	–	–	–	–	–	–	–	<1	–	1	–	<1	–	–	<1	–
Chenopodiaceae	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cistaceae																							
Cistus spp.	<1	<1	4	<1	–	<1	2	<1	<1	<1	1	<1	<1	<1			1	<1	<1	5	1	5	<1
Helianthemum spp.	<1	–	–	–	–	–	–	–	–	–	–	–	–	–			–	–	–	–	6	–	–
Cupressaceae																							
Cupressus/Juniperus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	<1	–	–	–	–
Ericaceae																							
Arbutus unedo	–	–	–	<1	<1	–	–	<1	–	–	–	6	–	1	<1	6	–	–	–	<1	–	<1	<1
Erica multiflora	6	<1	6	12	10	28	9	14	29	45	14	56	53	56	70	54	62	29	6	2	9	12	37
Euphorbiaceae																							
Euphorbia spp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	<1	–	–	–	<1	–	–	–	<1
Fabaceae																							
Acacia spp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Anthyllis cytisoides	–	–	–	<1	–	<1	–	–	–	–	–	–	–	4	–	–	–	–	<1	–	–	11	–
Astragalus spp.	–	–	–	–	<1	–	–	–	–	–	1	<1	–	<1	<1	<1	–	–	–	<1	–	2	–
Dorycnium pentaphyllum	–	–	<1	2	–	<1	2	–	–	–	1	<1	<1	1	2	<1	1	–	<1	2	9	<1	<1
Genista spp.	–	–	2	<1	–	–	2	–	2	–	–	–	–	<1	<1	<1	<1	–	<1	4	–	3	–
Lotus spp.	–	–	<1	<1	<1	–	<1	–	–	–	–	–	–	–	<1	–	<1	–	<1	3	–	–	–
Medicago spp.	–	–	2	<1	–	–	–	–	–	–	–	–	–	–	<1	–	–	–	–	–	–	–	–
Trifolium spp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Vicia spp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Fagaceae
Quercus ilex
Lamiaceae
Lavandula spp.
Micromeria inodora
Rosmarinus officinalis
Teucrium spp.
Thymus spp.
Liliaceae
Asparagus acutifolius
Asphodelus microcarpus
Mirtaceae
Eucalyptus spp.
Myrtus communis
Oleaceae
Olea europaea
Oxalidaceae
Oxalis pes-caprae
Palmae
Phoenix dactylifera
Pinaceae
Pyrus kalqarensis
Plantaginaceae
Plantago spp.
Poaceae
Ranunculaceae
Clematis cirrhosa
Rhamnaceae
Rhamnus alaternus
Rosaceae
Eriobotrya japonica
Rubus ulmifolius
Rutaceae
Citrus aurantium
Scrophulariaceae
Smilacaceae
Smilax aspera
Umbelliferae
Foeniculum vulgare

Note: < 1, values below 1%; — absence of the pollen type.

Unifloral honeys since ancient times, the carob tree grew in most countries of the Mediterranean basin; it is a characteristic species of the subtropical Mediterranean vegetation. Its value was recognised by the ancient Greeks, who brought it from Middle East to Greece and Italy and by the Romans and Arabs, who disseminated it along the North African coast and northwards into Spain and Portugal (Batlle & Tous, 1997; Ramón-Laca & Mabberley, 2004). Nowadays, themaincarobpopulations–spontaneous(feral)and cultivated – are concentrated predominantly in Spain and in the south of Italy (mainly in Sicily), Morocco, Greece, Cyprus, Turkey and Algeria. Ceratonia siliqua has an autumnal flowering (Retana et al., 1994; Bosch et al., 1996) and it is recognised as an important nectariferous and polliniferous species (Santas & Bikos, 1979; Ortiz et al., 1996). At the same time, it can provide honeydew (Damblon, 1988). In different honeys of the meridional territories of the Mediterranean such as Sicily, Turkey, the Iberian Peninsula and North Africa, the contents of Ceratonia pollen appears in varying

percentages, many times over-represented, due to the juxtaposition of the stamen and the nectariferous disc (Clemente & Salas, 1996; Terrab et al., 2001, 2002). Despite this, only a few honeys are known to be derived from the carob tree: Turkey (Ricciardelli d'Albore & Vorwohl, 1979), Monti Iblei in Sicily (Ricciardelli d'Albore, 1998), Morocco (Terrab et al., 2003a), and the Balearic Islands (Sáenz Laín & Gómez Ferreras, 2000). Ricciardelli d'Albore and Intoppa (2000) declare that the unifloral Ceratonia siliqua honeys are very rare and have a delicate smell, yellow colour and bitter taste. These characteristics are not comparable to those from the honeys in our study, which have a medium-high total pollen content, with a high percentage of C. siliqua pollen (57%), with amber or dark colour and infrequent bitter taste (probably due to the lack or rare presence of Arbutus unedo). The presence of Ericaceae in Ceratonia siliqua honey determines the reduction in electrical conductivity values (Guyot et al., 1999; Martins et al., 2008); this trend also appears in the studied samples of C. siliqua honey. The high electrical conductivity values in C. siliqua honeys analysed and the absence or almost absence of honeydew characteristics (HDE index <0.09%), which reveal the circumstantial value of C. siliqua as a source of honeydew, have been verified by some other authors (Damblon, 1988; Terrab et al., 2003b).

Table IV. The main volatile phenolic components in Ceratonia siliqua and Erica multiflora honeys (in %).

Table IV. The main volatile phenolic components in *Ceratonia siliqua* and *Erica multiflora* honeys (in %).

Phenolic compounds	Ceratonia siliqua	Erica multiflora
(e)-β-damascenone	1.3	0.0
2-acetil-5-methylfuran	0.5	0.0
2-cyclohexen-1-one	0.8	0.0
2-methylbutanoic acid	0.6	0.0
3-phenylfuran	0.0	1.2
α-pinene	0.5	3.7
camphene	0.2	2.8
epoxylinalool	1.1	0.0
1-(2-furanyl)-ethanone	0.2	0.0
hotrienol	10.4	0.0
limonene	0.0	5.4
linalool	0.6	0.0
cis-linalool oxide	6.6	0.0
trans-linalool oxide	57.7	13.3
oxoisophorone	0.0	3.4
p-menthane-1,8-diol	0.0	11.1
safranal	0.0	9.7

The other unifloral important honey is the Erica multiflora honey, an autochthon abundant plant in Mallorca and Eivissa, which flowers generously in autumn. It is recognised as an important nectariferous and polliniferous species predominant in the unifloral honey having an average of pollen of 50%, with a toffee or brown tonality rarely accompanied by Arbutus unedo. The electrical conductivity values in the Erica multiflora honey are variable; higher levels correspond to those honeys with more content of Ceratonia pollen, as it occurs with other Erica species honeys (400–700 µS/cm; Persano Oddo & Piro, 2004; Martins et al., 2008). Hedera helix honey, which can be found in Mallorca, is an obviously special unifloral honey from the Tramuntana Mountains. Hedera helix is cultivated in gardens or grown in feral form in humid

and rocky mountain sites. Hedera helix honey has high electrical conductivity values, although less than the Ceratonia siliqua or Erica multiflora honeys. In H. helix honey, the significant presence of pollen of C.siliqua and E.a multiflora is continuous.The sample of H.helixhoney detected a sensory characteristic different from the ones studied in Piana et al. (1989). Differences were discovered within the various amounts of volatile compounds observed depending upon the floral source of honey. Ceratonia siliqua honey volatile composition is characterised by a predominance of linalool and especially, its derivatives cis-linalool oxide, trans-linalool oxide and hotrienol, with a total amount of 75.3%. These values coincide with those of analytical data concerning nectar volatile composition of flowers analysed in several C. siliqua varieties studied (Custódio et al., 2006). However, this predominance is also characteristic in different honeys from tropical lands, as the ones from tahonal (Viguiera dentata Blake), tzitzilché (Gymnopodium floribundum Rolfen) in Yucatán (Cuevas-Glory et al., 2012) or from other climatic areas, such as the ones from corontillo [Escallonia pulverulenta (Ruiz et Pav.) Pers.] in Chile (Montenegro et al., 2009). In the Mediterranean context, these fruity aromatic compounds can be considered as typical markers of this unifloral honey. Erica multiflora honey shows a more diverse and balanced chemical volatile composition with six major represented compounds (46.6% of the total): α-pinene, limonene, trans-linalool oxide, pmenthane-1,8-diol, oxoisophorone, and safranal. Safranal and oxoisophorone can be considered as volatile markers of this honey. Isophorone, a 3,5,5trimethyl-cyclohex-2-ene-family derivative, was considered a reliable contributor in order to authenticate other honeys such as Calluna (Guyot et al., 1999) or Salix (Jerkovi´c & Marijanovi´ c, 2010). The presence of trans-linalool oxide could be related to the influence of Ceratonia siliqua.

Conclusions

Three unifloral honeys are recognised: carob (Ceratonia siliqua), winter heather (Erica multiflora) and ivy (Hedera helix). These species, which provide both nectar and pollen, are essential for bee nutrition during autumn (Lavie, 1975; Ortiz et al., 1996; Persano Oddo et al., 2004). The pollen of nectariferous species is dominant (78%). Ceratonia siliqua is the most important species of the autumnal honeys in the Balearic Islands and its presence is widespread in all the samples. Erica multiflora is a wild, natural, important and abundant autumnal plant. In general, autumnal honeys have a medium pollen component (pollen density between 4438 and 135500grains/g, average 18484). However, they have a narrow spectrum of pollen types with a limited number of species that bloom at this time of the year. High pollen levels of of Ceratonia siliqua and E. multiflora, in association with a few types such as Diplotaxis erucoides, Dittrichia viscosa, Phoenix sp. and Rosmarinus officinalis, distinguish the honeys from the Balearic Islands, characterised by other taxa typical in the Mediterranean region. These species differ to other honeys in the Mediterranean region like Morocco or Italy. The pollen can differentiate the honeys with origin in the Balearic Islands from those harvested in other latitudes. However, the presence of Micromeria inodora in the honeys of Eivissa is remarkable. This species can be considered as a guide species for the honeys obtained in the Pityusic Islands. It flowers in June–August and is very important for the survival of the bees. The volatile components are useful to distinguish different honeys. The Ceratonia siliqua honey has as a main feature a high presence of linalool and its derivatives, while Erica multiflora honey is distinguished by the abundance of p-menthane-1,8-diol and safranal. Phenolic composition obviously shows an exclusive presence of high values of trans-linalool

oxide in the C. siliqua honey and exclusive presence of hotrienol and cis-linalool oxide, whereas in Erica honey the particular compounds are p-menthane1,8-diol, safranal, limonene and α-pinene.

Acknowledgements

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