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Palynological and geographical characterization of avocado honeys in Spain

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The present work refers to the pollen analysis of 12 avocado honey samples from Spain. The samples were directly provided by the beekeepers, all professionals. The quantitative analysis showed that nectar is the main honey source in the samples studied, and that most honeys have a medium presence of botanical elements (BE); one sample belong to Class I of Maurizio, seven to Class II and four belong to Class III. The qualitative analysis of the samples showed the presence of 56 taxa belonging to 36 families. The Spanish avocado honeys are characterised by their medium content in pollen grains (NPG; $\bar{x} = 117000$) and their low honeydew indicator elements content (HDE; $\bar{x} = 6340$). *Echium plantagineum* gr. and *Genista* f. (present in 90% of the samples), and *Eucalyptus* f., *Olea europaea* L., *Mentha aquatica* gr. and *Reseda luteola* gr. (present in 80% of the samples), could be mentioned among the characteristic accompanying species of this honey type. The avocado honeys from the Iberian Peninsula and from the Canary Islands can be differentiated by the presence of *Asphalthium bituminosum* Medic., *Cardiospermum grandiflorum* Sw., *Phoenix canariensis* Hort. ex Chabaud and *Tropaeolum majus* L. in the latter.

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Usually, a honey is considered mainly from one plant (unifloral) if the pollen frequency of that plant is >45%. Some pollen grains, such as *Citrus* L., *Tilia* L. and *Lavandula* L., are under-represented in the honey spectra. On the contrary, other pollen types are over-represented (*Eucalyptus* L'Hér., *Castanea sativa* Miller, *Cynoglossum officinale* L. and *Myosotis* L.), and a honey in that group is regarded as unifloral only when the predominant pollen type represents more than 90% of the total (Maurizio 1979).

There are many factors influencing the under or overrepresentation of pollen in honey and these include: the size of pollen grains, large (e.g. *Acacia* Miller, *Agave* L., *Cucurbita* L., *Musa* L., *Opuntia* Miller, etc.) or small (e.g. *Alkanna* Tausch, *Castanea* Miller, *Eucalyptus* L'Hér., etc.), insufficient or lack of pollen (*Citrus* L., *Lavandula* L., *Salvia* L., etc.), monoecious and dioecious plants (*Asparagus* L., *Cucumis* L., Palmae, etc.), dominant pollen of nectarless species (Cistaceae, *Hypecoum* L., Gramineae, *Quercus* L., etc.). Due to all these factors, pollen analysis may not be enough in some cases to establish the unifloral nature of the honey. Thus the use of the sensory and physicochemical analysis becomes necessary. For example, the *Musa* honey can be considered as unifloral with less than 2% of pollen from this taxon (Ricciardelli D'Albore 1998).

In Spain and other Mediterranean countries some unifloral honeys have been the subject of many studies, for both their pollen contents and physicochemical properties these include; eucalyptus, orange, sunflower, lavender, heather, rosemary and honeydew honeys, among others (Accorti et al. 1986, Andrade et al. 1999, Kirkwood et al. 1960, Pérez-Arquillué et al. 1994, Persano Oddo et al. 1995, Ricciardelli D'Albore & Vorwohl 1979, Roselló Caselles et al. 1996, Serra Bonvehí 1988, 1989, Serra Bonvehí & Ventura Coll 1995, Terrab et al. 2001, 2003*a*, Thrasyvoulou & Manikis 1995).

On the other hand, there are few or no studies on some honey types, for example, alfalfa, viper's bugloss, buckthorn, carob tree, clover, crucifer, fruit trees, mint, wood sage, or willow honeys (Abu-Tarboush et al. 1993, Herrero et al. 2002, Pérez-Arquillué et al. 1995, Terrab et al. 2003 *b*). Avocado honey is one of the less studied types, with Israel and Spain being the main producers in the Mediterranean area. Avocado cultivation was introduced to Spain in the 1980's, with Granada, Málaga and Canary Islands being the main producing regions. Melissopalynological studies of this honey type are very scarce and only studies from the Canary Islands could be found in the literature (Orantes et al. 2002, Ramos et al. 1998).

Due to the large amounts of avocado honey produced in Spain and the appreciation of this honey by the consumers (Sáinz Laín & Gómez Ferreras 2000), as well as its scientific interest, the geographical characterization of this honey type is important. The goal of this work is to characterize Spanish avocado honeys, as well as to establish geographical markers that may help to distinguish the honeys of this region from those with a different geographical origin.

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MATERIAL AND METHODS

In this work we present the results of the pollen analysis of 12 samples of avocado honey collected between 2002 and 2003. These honeys show sensorial characteristics that allow them to be classified as unifloral of avocado with a fluid, bright, slightly opaque nature, and a very dark brown color. Samples were directly collected from professional beekeepers (Appendix & Fig. 1). The extraction of the honeys was made by centrifugation.

Quantitative analysis of samples follows the method described by Maurizio (1979) using the light microscope (LM) on slides prepared without any chemical treatment. All pollen grains (PG) and honeydew elements (HDE) were counted for each honey sample in four different slides, covering the whole surface of each slide. Botanical elements (BE) represent the addition of pollen grains and honeydew elements. Qualitative analysis used a 10 g subsample and acetolysed slides were prepared according to the method described by Erdtman (1960) but with modifications suggested by Hideux (1972). Following the results obtained by Behm et al. (1996), at least 500 pollen grains were counted among four different slides for each honey sample. Due to the fact that the pollen of *Persea americana* Mill. and the Lauraceae family in general are destroyed with the acetolysis process; the counting of these pollen grains for the spectra was made on natural preparations. Different pollen types were identified using the key published by Díez (1987) as the main source, but reference slides from the collection at the Department of Botany, University of Seville, were also used. Nomenclature for pollen types follows Persano Oddo & Ricciardelli D'Albore (1989). When possible identification to a specific level (e.g. *Ridolfia segetum*) was made, in other cases, to the generic level (e.g. *Echium plantagineum* gr.), or to a family level. Field observations made *in situ* while collecting the samples were also considered and, in these cases, the most likely species are quoted in brackets after the pollen type (e.g. *Genista* f. (*Chamaecytisus proliferus, Stauracanthus genistoides, Retama sphaerocarpa*)). Information on the different pollen and nectar sources in this study was authenticated by the authors in the field or recorded from literature (Herrera 1985, López et al. 1999, Ricciardelli D'Albore 1998, Rodríguez et al. 1999, Talavera et al. 1988).

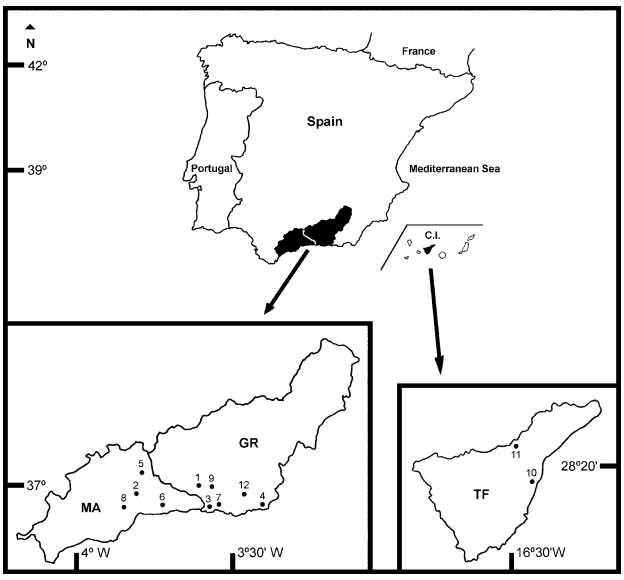

Fig. 1. Distribution of the honey samples studied (GR – Granada Province; MA – Málaga Province; TF – Tenerife Province; C.I. – Canary Islands; 1–12 – Collection sites, for identification see Appendix).

RESULTS

Results from the quantitative analysis (Table I) show that one sample was very poor in botanical elements (Class I) (Maurizio 1979), and 11 samples show a medium to medium-high content (Class II and III). The number of pollen grains (NPG) in 10 g of honey ranges between 11330 (sample 4) and 470100 (sample 11). Although all the samples show the presence of honeydew elements (HDE; fungic mycelium and spores), generally speaking the levels are low, ranging from 1000 (sample 1) to 20000 (sample 11).

Results from the qualitative analysis (Table II) illustrate the pollen types detected and their corresponding percentages in the different samples. Fifty six pollen types corresponding to 36 families have been identified. The best represented families in the samples studied are Fabaceae (with six types), Asteraceae, Cistaceae (five types), Apiaceae and Solanaceae (three types). Brassicaceae, Lamiaceae, Scrophulariaceae are represented with two types, and the rest of the families with only one type.

The families present with a higher frequency are: Cistaceae, Fabaceae and Lauraceae (present in 100% of the samples), Asteraceae and Boraginaceae (90%), Lamiaceae, Myrtaceae, Oleaceae and Resedaceae (85%), Brassicaceae, Papaveraceae and Scrophulariaceae (75%), and Caesalpiniaceae (65%).

The only pollen type present in all the samples is *Persea americana* Mill., with percentages ranging between 2% and 14%. *Echium plantagineum* gr., and *Genista* f. are present in 90% of the samples, with percentages between 1% and 51%. *Eucalyptus* f., *Mentha aquatica* gr., *Olea europaea* L. and *Reseda luteola* gr. are present in 85% of the samples, with percentages lower than 10%. In 75% of the samples the following types are present: *Calendula* f., ranging between $<1\%$ and 6%; *Cistus monspeliensis* L., less than 11%; *Ononis* f., between 2% and 12%; *Papaver* f., lower than 3%. *Ceratonia siliqua* L., *Cistus ladanifer* L. and *Halimium* f. are present in 64% of the samples, with percentages lower than 32%; *Kickxia* f., *Raphanus* f., and *Senecio* f. are present in 60% of the samples, with values ranging between $<1\%$ and 60%. In two samples the following species are present

Table I. *Summarized results of the quantitative analysis.*

Data refer to 10 g of honey. NPG – number of pollen grains; HDE – number of honeydew indicator elements; BEN – number of botanical elements.

Sample	NPG	HDE	BEN	Class
1	40 600	1000	41 600	II
2	35 100	3400	38 500	II
3	49 200	4400	53 600	II
4	11 330	2400	13 730	I
5	104 100	6300	110 400	III
6	95 800	3780	99 580	II
7	169 000	14 500	183 500	III
8	148 900	3600	152 500	III
9	92 800	4200	97 000	II
10	85 530	7930	93 460	II
11	470 100	20 000	490 100	III
12	71 520	4600	76 120	II

Table II. *Results of the qualitative analysis, represented as percentages.*

(+) – values below 1%; (–) – absence of the pollen type; B.V. – beekeeping value; A – anemophilous plants; N – nectariferous plants; NL – nectarless plants.

Pollen type	1	2	3	4	5	6	7	8	9	10	11	12	B.V.
Apiaceae													
Eryngium f.	+	–	–	–	–	–	–	–	–	–	–	+	N
Pinpinella villosa	+	–	+	–	2	–	–	–	1	–	–	–	N
Ridolfia segetum	–	–	–	–	–	–	–	–	–	–	1	–	N
Apocinaceae													
Nerium oleander	8	–	–	–	–	–	–	–	2	–	–	–	NL
Aquifoliaceae													
Ilex canariensis	–	–	–	–	–	–	–	–	–	3	5	–	N
Asteraceae													
Arctotheca calendula	–	–	–	–	+	–	–	–	–	–	–	–	NL
Calendula f.	5	–	2	2	+	–	2	6	–	1	2	–	NL
Crepis f.	–	–	–	–	+	–	–	–	–	+	1	–	NL
Lactuca f. (*Scolymus hispanicus*, *S. maculates*)	–	–	–	–	–	–	+	–	–	–	–	+	NL
Senecio f. (*Onopordum dissectum*, *Silybum marianum*)	–	3	3	1	1	–	–	2	20	–	–	1	NL
Boraginaceae													
Echium plantagineum gr. (*E. plantagineum*)	1	27	2	3	3	8	38	15	3	32	–	1	N
Brassicaceae													
Capsella f.	–	–	–	6	–	–	3	–	–	–	–	+	N
Raphanus f.	–	1	–	–	–	3	–	4	1	6	1	–	N
Caesalpiniaceae													
Ceratonia siliqua	3	5	–	2	3	3	5	6	–	–	–	–	N
Campanulaceae													
Campanula erinus	3	–	–	–	–	–	–	–	–	–	–	–	NL
Caryophyllaceae													
Silene vulgaris gr.	5	–	–	+	+	–	–	–	–	–	–	–	N
Cistaceae													
Cistus ladanifer	–	–	1	4	1	11	3	1	–	–	+	1	NL
Cistus monspeliensis	–	6	22	–	3	6	9	4	11	1	–	3	NL
Cistus salvifolius	–	6	6	–	–	–	6	–	–	–	–	3	NL
Halimium f. (*H. halimifolium*)	28	–	–	32	7	–	–	2	5	+	7	–	NL
Helianthemum f.	–	–	–	–	–	–	–	–	–	+	–	–	NL
Cucurbitaceae													
Cucurbita f.	–	+	–	–	–	–	–	–	–	1	–	–	N
Ericaceae													
Erica sp.	1	–	–	–	–	2	–	–	–	5	7	+	N
Fabaceae													
Asphalthium bituminosum	–	–	–	–	–	–	–	–	–	1	1	–	NL
Genista f. (*Chamaecytisus proliferus, Stauracanthus genistoides*, *Retama sphaerocarpa*)	18	24	40	15	51	13	12	24	40	12	–	3	NL
Onobrychis f. (*O. peduncularis)*	–	–	–	–	–	–	–	–	–	+	–	–	N
Ononis f. (*O natrix*)	5	8	2	2	12	2	3	5	–	–	–	1	NL
Psoralea americana	–	–	–	–	–	–	–	–	–	1	–	–	NL
Trifolium arvensis gr.	–	–	–	–	1	–	–	–	–	–	–	–	N
Fagaceae													
Quercus f. (*Q. suber*)	–	–	2	–	–	7	–	2	–	1	1	4	A
Fumariaceae													
Hypecoum f.	–	–	+	–	–	–	–	–	+	–	–	–	NL
Geraniaceae													
Tropaeolum majus	–	–	–	–	–	–	–	–	–	+	1	–	NL
Lamiaceae													
Mentha aquatica gr. (*M. pulegium*)	3	+	+	+	1	6	1	2	+	–	–	1	N
Teucrium scorodonia gr.	–	–	–	1	–	–	–	–	–	–	–	–	N
Lauraceae													
Persea Americana	7	13	7	4	2	2	4	14	7	12	2	6	N
Liliaceae													
Muscari f.	–	–	–	1	–	–	–	–	–	–	–	–	N
Lythraceae													
Lythrum salicaria gr. (*L. junceum*)	–	–	–	+	–	–	–	–	–	–	–	–	N
Mimosaceae													
Acacia f.	–	–	–	–	–	–	+	–	–	–	–	–	N
Myrtaceae													
Eucalyptus f. (*E. camaldulensis*, *E. gomphocephala*)	3	1	2	10	6	–	6	3	1	+	–	+	N

Table II. *(Continued)*

Pollen type	1	2	3	4	5	6	7	8	9	10	11	12	B.V.
Oleaceae													
Olea europaea	–	1	4	–	+	1	2	4	4	2	1	1	A
Palmaceae													
Phoenix canariensis	–	–	–	–	–	–	–	–	–	3	6	–	A
Papaveraceae													
Papaver f.	3	3	2	3	–	2	–	+	3	2	–	+	NL
Pinaceae													
Pinus sp.	–	–	–	–	–	–	–	–	–	+	–	–	A
Plantaginaceae													
Plantago f. (*P. coronopus*)	–	–	–	–	+	–	–	–	–	–	–	–	A
Poaceae													
Poacea > 37 μm	–	–	–	–	–	–	+	1	–	–	–	–	A
Resedaceae													
Reseda luteola gr.	–	+	1	2	5	–	2	4	1	9	1	1	NL
Rhamnaceae													
Rhamnus alaternus	–	–	–	2	–	–	–	+	–	–	–	–	N
Rosaceae													
Fragaria f.	–	–	–	–	–	+	+	–	–	+	–	–	NL
Salicaceae													
Salix fragilis gr.	–	–	–	1	–	3	2	–	–	–	–	+	N
Sapindaceae													
Cardiospermum grandiflorum	–	–	–	–	–	–	–	–	–	+	+	–	NL
Scrophulariaceae													
Kickxia f.	6	+	2	5	–	30	–	–	–	–	60	72	A
Verbascum f. (*V. sinuatum*)	–	–	–	–	–	–	–	+	–	3	+	+	A
Solanaceae													
Hyoscyamus albus	–	–	–	1	–	–	–	+	–	1	–	–	NL
Solanum dulcamara	–	–	–	–	–	–	–	–	–	–	+	–	NL
Solanum nigrum	–	–	–	–	–	–	–	–	–	2	–	–	NL
Thymelaeaceae													
Thymelaea f. (*Daphne gnidium*)	+	–	–	–	–	–	–	–	–	–	–	–	N

Asphalthium bituminosum Medic., *Cardiospermum grandiflorum* Sw., *Cucurbita* f., *Eryngium* f., *Hypecoum* f., *Lactuca* f., *Ilex canariensis* Poir., *Nerium oleander* L., Poaceae >37 μm, *Phoenix canariensis* Hort. ex Chabaud, *Rhamnus alaternus* L. and *Tropaeolum majus* L., all with very low percentages (<5%), except for *Nerium oleander* L. and *Phoenix canariensis* Hort. ex Chabaud with 8% and 6% respectively. The rest of the pollen types are present only in one sample, with low percentages never higher than 3%.

DISCUSSION

The avocado honeys of Spain are derived from *Persea americana* Mill. This is an important species in nectar production, and has a flowering period between February-March to June-July. The percentage of *Persea americana* pollen (Table I) ranges between 2% (samples 5, 6 and 11) and 14% (sample 8). These are low percentages when compared with other authors' studies (e.g. Ramos et al. 1998). The low percentages are probably due to avocado flowering behavior, which can be termed diurnally synchronous protogyny dichogamous, with intermediate closing. The bisexual flower opens twice, at the first opening it acts as a female, and at the second opening, usually on the next day, it acts as a male and pollen is released. The opening and the closing of the female stage flowers of a single tree (or cultivar), as well as that of the male stage flowers, occur simultaneously, each of the two flowering stages occurs during different parts of the day. All the avocado types are divided to two complementary flowering groups. 'Group A' types bear, in a warm weather, open female stage flowers from the morning to noon time, and male stage flowers during the afternoon. 'Group B' types, on the other hand, bear male stage flowers in the morning and open female stage flowers in the afternoon. These male and female flowering phases overlap for a daily period of one to three hours in some of the avocado cultivars. Under cool weather conditions there is a delay of the male and the female openings, which may result in a complete reversal in the part of the day female and male flowers are open. At both male and female openings nectar is secreted, thus, insects that collect nectar, or nectar with pollen, are the potential pollinators of the avocado (Davenport 1986, Ish-Am & Eisikowitch 1992, Stout 1923). Furthermore, some observations and experiments have demonstrated a very low and inefficient rate of avocado pollen transfer through the hive by body contact among the honey bees (Ish-Am & Eisikowitch 1998).

The low percentages of *Persea americana* pollen greatly increase (ranging between 5% in sample 6 and 60% in sample 12) when pollen grains from anemophilous and nectarless plants (see Table II) are excluded in calculations of percentages (Table III). The avocado honey type is

characterized by medium pollen content (mean NPG = 117000), 66% of the honeys belong to Class II of Maurizio and 33% to Class III. They are also characterized by a low HDE content (= 6340). The most characteristic accompanying species are *Echium plantagineum* gr. and *Genista* f. (present in more than 90% of the samples), and *Eucalyptus* f. *Olea europaea* L., *Mentha aquatica* gr. and *Reseda luteola* gr. (in more than 80% of the samples).

Few studies have characterized avocado honey from other countries. According to the results obtained here (Table II), honeys from the Iberian Peninsula (Granada and Málaga) can be differentiated from those in Tenerife (Canary Islands) due to the absence of some pollen types. Pollen grains from *Asphalthium bituminosum* Medic., *Cardiospermum grandiflorum* Sw., *Phoenix canariensis* Hort. ex Chabaud and *Tropaeolum majus* L. occur in avocado honey from the Canary Islands but are lacking from Iberian Peninsula honeys as the parent plants do not occur in the latter region.

The only studies on this honey type were based on Spanish honeys (Ramos et al. 1998, Orantes et al. 2002). These authors studied several honey samples from La Palma (Canary Islands), but only one sample of avocado honey was detected by each author. Both showed medium pollen content (7333 grains/g of honey) and a medium pollen types (26 pollen types). These two samples showed *Castanea sativa* Mill., *Prunus dulcis* (Mill.) D.A.Webb, *Rumex* sp. and *Euphorbia obtusifolia* Lam. as the most characteristic accompanying species, which can differentiate them from the two samples (10 and 11) from Tenerife; they also showed other accompanying species such as *Echium* sp., *Erica* sp., Brassicaceae, *Cistus* L. and *Chamaecytisus proliferus* Link, also present in the two samples from the Canary Islands in this study.

CONCLUSIONS

The pollination biology of the avocado flowers make it difficult to distinguish this honey type based on the pollen honey spectra, especially as pollen grains of *Persea americana* are frequently destroyed during acetolysis. Thus, it is recommended the counting of pollen grains from avocado honeys be based on natural preparations.

Table III. *Percentage of Persea americana pollen in each sample after eliminating the pollen grains from anemophilous and nectarless plants.*

Sample	% *Persea* pollen
1	29
2	27
3	54
4	13
5	11
6	5
7	7
8	34
9	58
10	21
11	16
12	60

Also, it is essential to carry out a previous sensorial analysis to certify the floral origin of this honey type, since the avocado pollen percentage may not surpass 2%. The low pollen representation is due to the special characteristics of avocado plants, which have a short flowering period, low pollen production and very large pollen grains. Furthermore the knowledge that bees tend to remove many of these pollen grains from avocado nectar before returning to the hive makes the verification of this type of honey particularly difficult.

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APPENDIX. COLLECTION SITES

1. Otívar (Granada);
2. Benamargosa (Málaga);
3. Almuñecar (Granada);
4. Calahonda (Granada);
5. Periana (Málaga);
6. Frijiliana (Málaga);
7. Almuñecar (Granada);
8. Almáchar (Málaga);
9. Otívar (Granada);
10. Güimar (Tenerife);
11. Santa Úrsula (Tenerife);
12. Motril (Granada).

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