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Physicochemical attributes and pollen spectrum of some unifloral Spanish honeys

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In the present work a total of 19 unifloral Spanish honeys were studied and botanically typified: willow (*Salix* sp.), sainfoin (*Onobrychis viciifolia* Scop.), chickweed (*Hypecoum* sp.), crucifer (*Brassica* type), fruiter (*Prunus* sp.), thyme (*Thymus* sp.), blueweed (*Echium* sp.), spike lavender (*Lavandula latifolia* Med.), French lavender (*Lavandula stoechas* L.), and vetch (*Vicia sativa* L.). Unifloral honeys were considered as such whenever the dominant pollen was found at over 45% of total pollen, except for lavender and thyme types of honey where a finding above 15% of *Lavandula* or *Thymus* pollen, respectively, was enough to typify them. All samples were organoleptically examined and the following determinations were carried out: moisture, optical rotation, electrical conductivity, ash, hydroxymethylfurfural, diastase activity, pH, acidity (free, lactone, and total) and carbohydrate composition. These samples were found to meet all major national and international honey specifications.

INTRODUCTION

Honey production in Spain during 1991 (21 000 Tm) was the first and largest among European countries and totalled 12% of honey production in Europe (FAO, 1992). From a wide spectrum of different flowers available to honey bees, 11 main kinds of essentially unifloral honeys can be obtained in our national market (Ortega, 1987). Sunflower honey (*Helianthus annuus* L.) constitutes the largest production followed by rosemary honey (*Rosmarinus officinalis* L.). Several works have been done on the most important Spanish unifloral honeys (Serra, 1988; Mateo *et al.*, 1992; Gómez *et al.*, 1993; Serra & Ventura, 1993; Pérez-Arquillué *et al.*, 1994). These authors reported the physical properties and chemical composition of some organoleptically interesting honeys which were compared with other types imported from around the world. However, a lot of other high-quality unifloral honeys produced in small amounts by local bee-keepers are beginning to be marketed and their quality needs evaluation. In Spain there are general regulations for honey, but not for the different unifloral honeys, which are the types in demand. This is also emphasised by the fact that honey consumption per person in Spain has increased considerably in recent years, doubling from 0·3 kg/year in 1984 to 0·7 kg/year in 1992 (Crane, 1990; MAPA, 1993).

Therefore, the present work was conducted to investigate the quality of 10 types of Spanish unifloral honeys produced on a small scale, though most of them are broadly appreciated by consumers.

MATERIALS AND METHODS

Sample collection and floral-type identification

Nineteen samples of honey collected from bee-keepers were classified according to their botanical origin using the method of Louveaux *et al.* (1978) as described elsewhere (Pérez-Arquillué *et al.*, 1994). The 10 honey types identified were the following (Table 1): willow (*Salix* sp.), sainfoin (*Onobrychis viciifolia* Scop.), chickweed (*Hypecoum* sp.), crucifer (*Brassica* sp), fruiter (*Prunus* sp.), thyme (*Thymus* sp.), blueweed (*Echium* sp.), spike lavender (*Lavandula latifolia* Med.), French lavender (*Lavandula stoechas* L.), and vetch (*Vicia satila* L.). Botanical classification was achieved when the pollen spectrum contained >45% of the corresponding dominant pollen. However, the pollen spectrum of lavender and thyme honeys often contains as little as 15% of *Lavandula* and *Thymus* pollen, respectively. Maturity, purity, deterioration, and adulteration criteria indicated that the quality of the honeys was good and all samples showed no signs of fermentation or granulation.

Physicochemical analysis

The samples of honey were analysed according to the

Table 1. Pollen analysis of 10 different types of Spanish unifloral honeys

Honey type	*n*	Pollen analysis of sediment
Willow (*Salix* sp.)	4	***Salix* sp.**, *Rosmarinus officinalis, Vicia faba, Genista* type, *Diplotaxis erucoides, Prunus* sp.
Sainfoin (*Onobrychis viciifolia* Scop.)	4	***Onobrychis viciifolia***, *Thymus* sp., *Salix* sp., *Lavandula* sp., *Trifolium repens, Centaurea jacea, Cistus* sp.
Chickweed (*Hypecoum* sp.)	2	***Hypecoum* sp.**, *Rosmarinus officinalis, Onobrychis viciifolia, Helianthemum* sp., *Genista* type, *Dorycnium* sp.
Crucifer (*Brassica* sp.)	2	***Brassica* sp.**, *Diplotaxis erucoides, Thymus* sp., *Helianthus annuus, Genista* type, *Taraxacum officinale*
Fruiter (*Prunus* sp.)	2	***Prunus* sp.**, *Onobrychis viciifolia, Salix* sp., *Thymus* sp., *Genista* type, *Diplotaxis erucoides*
Thyme (*Thymus vulgaris* L.)	1	***Thymus* sp.**, *Rosmarinus officinalis, Prunus* sp., *Helianthus annuus, Onobrychis* sp., *Genista* type, *Quercus* sp.
Blueweed (*Echium* sp.)	1	***Echium* sp.**, *Helianthus annuus, Lavandula stoechas, Cistus* sp., *Taraxacum officinale, Erica* sp., *Thymus* sp.
Spike lavender (*Lavandula latifolia* Med.)	1	***Lavandula latifolia***, *Thymus* sp., *Dorycnium* sp., *Onobrychis* sp., *Carduus/Cirsium, Centaurea cyanus*
French lavender (*Lavandula stoechas* L.)	1	***Lavandula stoechas***, *Echium* sp., *Campanula* sp., *Helianthus annuus, Satureja montana, Quercus* sp.
Vetch (*Vicia sativa* L.)	1	***Vicia sativa***, *Diplotaxis erucoides, Rosmarinus officinalis, Carduus/Cirsium, Prunus* sp., *Onobrychis* sp.

official Spanish methods (BOE, 1986) and the AOAC methods (AOAC, 1990) so as to determine moisture, optical rotation, electrical conductivity, ash content, hydroxymethylfurfural (HMF), diastase activity, pH, acidity (free, lactone, and total) and carbohydrate composition. Two replicate analyses were made from each sample to obtain the reported data.

Moisture in honey was determined with a Shibuya refractometer reading at 20°C and obtaining corresponding % moisture from the Chataway table (Chataway, 1935), revised and updated (BOE, 1986; AOAC, 1990; Crane 1990).

Optical rotation was measured in a polarimeter (Carl Zeiss 811753) as follows: 10 g of honey sample was clarified with Carrez reagents (I and II) and distilled water was added to get a final volume of 100 ml. Then, this solution was inserted into the polarimeter and results were read in angular degrees on a 200 mm basis.

Electrical conductivity of a honey solution at 20% (dry matter basis) in CO_2-free deionised distilled water was measured at 20°C in a Crison 522 conductimeter. Results were expressed as $10^{-4}S \times cm^{-1}$ (BOE, 1986).

Ash percentage was measured by calcination, overnight at 550°C in furnace, to constant mass (BOE, 1986; AOAC, 1990).

Hydroxymethylfurfural was determined after clarifying samples with Carrez reagents (I and II) and addition of sodium bisulfite (based on methodology described in AOAC (1990). Absorbance was determined at 284 and 336 nm in a 1 cm quartz cuvette in a Kontron spectrophotometer. Results were expressed as mg/kg.

Diastase activity was measured using a buffered solution of soluble starch and honey which was incubated in a specially designed glass tube, shaped to end in an inverted 'V', in a thermostatic bath until the end-point was determined photometrically (Spectronic 20). Results were expressed (as Gothe degrees) as ml of 1% starch hydrolysed by an enzyme in 1 g honey in 1 h (AOAC, 1990).

pH was measured in a pH meter Crison 2001 from a solution containing 10 g honey in 75 ml of CO_2-free distilled water.

Free, lactone, and total acidity were determined as follows by the titrimetric method: addition of 0·05M NaOH is stopped at pH 8·50 (free acidity), immediately 10 ml 0·05M NaOH is pipetted in, and without delay back-titrated with 0·05M HCl to pH 8·30 (lactone acidity). Total acidity results from adding free plus lactone acidities (BOE, 1986; AOAC, 1990). Results were expressed as meq/kg.

The carbohydrate composition was determined by gas–liquid chromatography with flame ionisation detector (GLC–FID) based originally on the method by Pourtallier and Rognone (1977) modified by Serra and Bosch (1989). Trimethylsilyl derivatives of sugar oximes were baseline separated and quantitated in a gas chromatograph HP 5890 Series II and an HP 3396A integrator under the following conditions: 3 m stainless-steel column (1/8-in. o.d.) packed with 4% SE-52 on Chromosorb WAWDNCS 100/120 mesh, carrier gas flow 25 ml N_2/min, FID with H_2 at 30 ml/min and O_2 at 400 ml/min, temperatures (°C) injector 280, detector 290 and column 205, rate 2°C/min to 280°C, held for 20 min, internal standard calibration with xylose. All standard sugars were obtained from Sigma Chemical Company. Results were expressed as grams of each sugar in 100 g of honey (percentage).

Statistical analysis was designed using StatView™ SE+Graphics (Abacus Concepts, Inc., 1988, Berkeley, CA, USA).

RESULTS AND DISCUSSION

The results of the honey pollen analyses are shown in Table 1. The dominant pollen (>45% of pollen spectrum, except for lavender and thyme which are only

Table 2. Analysis of some physicochemical parameters (mean ± SD) in several types of Spanish unifloral honeys

Parameter	Honey types[a]									
	Willow	Sainfoin	Chickweed	Crucifer	Fruiter	Thyme	Blueweed	Spike lavender	French lavender	Vetch
Moisture (%)	16·70 ± 0·14	18·50 ± 1·73	17·20 ± 0·28	18·75 ± 0·78	17·50 ± 0·00	18·00 ± 0·45	16·40 ± 0·41	17·20 ± 0·43	16·00 ± 0·40	18·30 ± 0·46
Optical rotation $\alpha^{20°C}$	−1·05 ± 0·94	−1·46 ± 1·19	−0·92 ± 0·56	−15·07 ± 1·12	−4·73 ± 0·37	−11·10 ± 0·28	−4·27 ± 0·11	−1·97 ± 0·05	−3·14 ± 008	−1·95 ± 0·05
Electrical conductivity ($10^{-4}S \times cm^{-1}$)	1·59 ± 0·80	2·13 ± 0·23	1·63 ± 0·05	2·63 ± 0·03	1·39 ± 0·66	4·47 ± 0·11	3·96 ± 0·10	1·84 ± 0·05	1·66 ± 0·04	2·70 ± 0·07
Ash content (%)	0·06 ± 0·02	0·09 ± 0·02	0·05 ± 0·03	0·12 ± 0·01	0·23 ± 0·08	0·29 ± 0·01	0·18 ± 0·01	0·06 ± 0·01	0·07 ± 0·01	0·11 ± 0·01
HMF (mg/kg)	1·49 ± 1·00	3·32 ± 3·57	2·91 ± 0·32	4·38 ± 0·70	3·51 ± 2·86	25·9 ± 0·65	13·9 ± 0·35	0·98 ± 0·02	4·79 ± 0·12	10·7 ± 0·27
Diastase activity (G°)	16·9 ± 1·08	25·0 ± 8·89	17·1 ± 0·14	46·6 ± 4·88	30·9 ± 2·91	39·3 ± 0·98	15·8 ± 0·39	25·4 ± 0·63	10·8 ± 0·27	38·4 ± 0·96
pH (geometric mean)	3·68	3·84	3·65	3·65	4·11	4·24	0·04	3·54	3·74	3·94
Free acidity (meq/kg)	17·5 ± 0·58	17·2 ± 3·29	18·5 ± 2·12	22·0 ± 6·03	22·5 ± 0·00	26·9 ± 0·67	24·2 ± 0·61	26·2 ± 0·66	14·0 ± 0·35	19·7 ± 0·49
Lactone acidity (meq/kg)	0·44 ± 0·43	1·86 ± 1·50	1·25 ± 1·06	1·85 ± 0·18	3·38 ± 0·53	1·24 ± 0·03	2·75 ± 0·07	3·85 ± 0·10	1·25 ± 0·03	2·43 ± 0·06
Total acidity (meq/kg)	17·9 ± 0·66	19·0 ± 2·84	19·8 ± 1·06	23·9 ± 6·21	25·9 ± 0·53	28·1 ± 0·70	27·0 ± 0·67	30·1 ± 0·75	15·3 ± 0·38	22·1 ± 0·55

[a]Willow (*Salix* sp.), sainfoin (*Onobrychis viciifolia* Scop.), chickweed (*Hypecium* sp.), crucifer (*Brassica* sp.), fruiter (*Prunus* sp.), thyme (*Thymus vulgaris* L.), blueweed (*Echium* sp.), spike lavender (*Lavandula latifolia* Med.), French lavender (*Lavandula stoechas* L), vetch (*Vicia sativa* L.)

Table 3. Analysis of sugar composition (mean ± SD) in several types of Spanish unifloral honeys

Parameter	Honey types[a]									
	Willow	Sainfoin	Chickweed	Crucifer	Fruiter	Thyme	Blueweed	Spike lavender	French lavender	Vetch
Monosaccharides										
Fructose	33·7 ± 0·86	37·3 ± 1·47	35·0 ± 0·28	35·3 ± 0·71	36·9 ± 0·13	36·4 ± 0·91	40·0 ± 1·00	37·9 ± 0·95	40·5 ± 1·01	39·3 ± 0·98
Glucose	28·5 ± 0·74	31·6 ± 1·49	29·3 ± 0·64	38·1 ± 3·71	29·1 ± 0·15	30·0 ± 0·75	29·7 ± 0·74	32·1 ± 0·80	28·8 ± 0·72	31·6 ± 0·79
Disaccharides										
Sucrose	2·95 ± 0·84	1·35 ± 1·81	3·19 ± 0·51	0·07 ± 0·03	0·51 ± 0·06	0·39 ± 0·01	0·41 ± 0·01	1·09 ± 0·03	0·35 ± 0·01	0·67 ± 0·02
Maltose	7·49 ± 0·99	6·72 ± 0·63	7·70 ± 0·22	7·93 ± 1·36	7·85 ± 0·35	8·09 ± 0·20	7·30 ± 0·18	8·12 ± 0·20	6·69 ± 0·17	7·33 ± 0·18
Trisaccharides										
Erlose	0·41 ± 0·21	0·46 ± 0·05	0·66 ± 0·06	ND[b]	0·23 ± 0·04	0·74 ± 0·02	0·56 ± 0·01	0·60 ± 0·01	1·76 ± 0·04	0·51 ± 0·01
Melezitose	0·04 ± 0·01	0·05 ± 0·01	ND	ND	ND	ND	0·27 ± 0·01	0·25 ± 0·01	ND	ND
Ratio fructose to glucose	1·18 ± 0·05	1·18 ± 0·05	1·19 ± 0·02	0·93 ± 0·07	1·27 ± 0·01	1·21 ± 0·03	1·35 ± 0·03	1·18 ± 0·03	1·41 ± 0·04	1·24 ± 0·03

[a]Willow (*Salix* sp.), sainfoin (*Onobrychis viciifolia* Scop.), chickweed (*Hypecium* sp.), crucifer (*Brassica* sp.), fruiter (*Prunus* sp.), thyme (*Thymus vulgaris* L.), blueweed (*Echium* sp.), spike lavender (*Lavandula latifolia* Med.), French lavender (*Lavandula stoechas* L.), vetch (*Vicia sativa* L.)
[b]ND, none detected.

>15%) is quoted first (italised and boldfaced) followed by the most important accompanying pollen. Sporadic or minor pollen that appeared in less than 3% of pollen spectra are not listed in Table 1. The botanical families Labiatae Leguminosae, Cruciferae, and Rosaceae were most frequently found in the samples.

Tables 2 and 3 show the mean (±SD) results obtained from the physicochemical and carbohydrate composition analyses of honey samples, respectively. The parameters indicating product maturity gave values that fell within the limits set by current European Community and Spanish regulations. Thus, average moisture ranged from 16·00 to 18·75, while current EC regulations (Directive 74/409/EEC) require <21% moisture in honey for safety from fermentation; diastase activity was high, from 10·70 to 46·55 (Gothe degrees), and HMF values were very low (less than 15 mg/kg, except for a sample of thyme honey that was 25·88 mg/kg); both indicated the high degree of freshness of these honeys. EC regulations set a minimum diastase number of 8 on the Gothe scale, and a maximum HMF content of 40 mg/kg. The total acidity was likewise within limits (below 40 meq/kg) indicating absence of undesirable fermentation; the pH found in all samples (between 3·54 and 4·24) corresponded to that of floral honeys in contrast to somewhat higher pHs, around 4·5, which can be found in honeydew honeys (Crane, 1990). The electrical conductivity was low, below 4·0 $10^{-4}S \times cm^{-1}$ except for thyme honey which was 4·47 $10^{-4}S \times cm^{-1}$. The range of values for ash content (0·05–0·29%) fell within the limit allowed for floral honeys (0·6%) and indicated the cleanness of honey samples and possibly the lack of adulteration with molasses. Not only were all samples well within acidity and ash levels, but generally all samples were on the low side of the floral honey distribution for all acidity and ash values (Krauze & Zalewski, 1991). The figure obtained from adding the percentages of fructose, glucose plus maltose was above 65%, the minimum limit set by EC regulations for reducing sugars. The mean percentages of sucrose were all below 5%, which is the maximum limit proposed by FAO/WHO Standards of honey (Crane, 1990) and EC Directive 74/409. The percentages of trisaccharides erlose, and melezitose were low, in keeping with data reported by Serra *et al.* (1987) from different Spanish unifloral honeys. The fructose/glucose ratios were widely distributed (0·93–1·41) indicating the variety of floral sources whence the honey samples originated.

Willow honey (*Salix* sp.)

The willow is a quick-growing tree that belongs to the Salicaceae family and bears heavy-producing pollen male aments with honey yields between 101 and 200 kg/ha (Crane, 1975). Willow honey is light amber, mild-flavoured, has fine aroma and produces small crystals. Samples were levorotatory (α = –1·05), with 16·70% moisture which was less than that reported by Zurcher *et al.* (1975) in willow honey. Conductivity was below 3·0 $10^{-4}S \times cm^{-1}$, and both pH and ash content were low. Mean value of 1·49 mg/kg HMF together with an average diastase activity (16·86°G) gave a suitable freshness degree. Total acidity was less than half the Spanish and EC maximum limit of 40 meq/kg. The ratio fructose/glucose (F/G) was 1·18, sucrose averaged 2·95%, and melezitose 0·04%. Zurcher *et al.* (1975) reported a similar ratio F/G (1·15) and smaller sucrose proportion in total sugars whereas melezitose was not detected.

Sainfoin honey (*Onobrychis viciifolia* Scop.)

This leguminous plant is widely cultivated for animal feeding, and has a honey yield between 51 and 200 kg/ha. Louveaux and Vergeron (1964) reported the presence of pollen from Cistaceae, Labiatae, and *Hypecoum* sp. in sainfoin honey samples. This kind of honey is water-white and very light, very sweet, with more pronounced flavour than other legumes (Crane, 1975) and dense crystallisation of variable diameter at each given temperature (Piana *et al.*, 1989). It is a slightly levorotatory (α = –0·98), high-moisture type of honey (average 18·50%); one sample was above the Spanish maximum moisture limit (20%; BOE, 1983) but did not exceed the EC limit set to 21%. Conductivity and acidity may be considered low. Crane (1990) reported that sainfoin honey often contains less than 0·1% ash; 0·09% was the mean ash content found in our samples. Proper freshness degree was estimated from HMF (3·32 mg/kg) and diastase activity (24·99°G).

The ratio F/G was 1·18, higher than <1 reported by Battaglini and Bosi (1973) in sainfoin honey. However, sucrose and maltose, respectively, averaged 1·35% and 6·72%, both values well above those reported in Italian sainfoin honey (0·43% and 3·41%, respectively). As reported by those authors, melezitose averaged 0·81%, whereas our samples only reached 0·05%

Chickweed honey (*Hypecoum* sp.)

The pollen of this Papaveraceae often appears in Spanish rosemary honey, but in some cases can constitute a palynologically well-differentiated type of honey. This honey is white-coloured, light-flavoured and shows fine and fast crystallisation. Slightly levorotatory (α = –0·92), conductivity, ash content, diastase activity and pH gave normal values. The sucrose proportion (3·19%) was the highest among samples; the ratio F/G was 1·19, and erlose proportion (0·66%) can be considered relatively high.

Crucifer honey (*Brassica* sp.)

These honey samples were light amber, strong-flavoured and fast crystallising. Of the total sugars, fructose amounted to 35·28%, and glucose averaged 38·13%. Thus, the ratio F/G was less than 1 in good agreement with French rape honey (Pourtallier & Taliercio, 1970). Maltose averaged 7·93%, one of the highest, whereas

sucrose was detected in lesser amounts (0·07%); no trisaccharides were detected. Conductivity reached 2·63 10^{-4}S × cm^{-1}, just within the range 1·2–2·7 10^{-4}S × cm^{-1} proposed by Pourtallier and Taliercio (1970) for rape honey. Crucifer honey was the most levorotatory of all types examined (α = −15·07) and showed the highest moisture content (18·75%). pH was low (3·65) and diastase activity was very high (46·55°G).

Fruiter honey (*Prunus* sp.)

Honey bees usually suck nectar of fruiters (almond, peach, plumb trees) thus improving pollination. Fruiter honey samples analysed were amber, light-flavoured and fast crystallising in small crystals. Conductivity was low (1·39 10^{-4}S × cm^{-1}) and pH was 4·11. Lactone acidity was the highest detected in all samples (3·38 meq/kg). The ratio F/G was 1·27; sucrose proportion averaged 0·51% and melezitose was not detected.

Thyme honey (*Thymus* sp.)

This mint of the family Labiatae has great nectarial value and yields an amber honey, strong-flavoured with variable crystallisation (Piana *et al.*, 1989). Louveaux and Vergeron (1964) mentioned that Spanish thyme honey often contains pollen of Cistaceae giving typical mixtures with *Thymus* sp., *Hypecoum* sp., *Ulex* sp., and pollen from fruiters.

Both conductivity and ash content may be considered high. Thyme honey samples were clearly levorotatory (α = −11·10). According to Crane (1975) enzymatic activity is high in this kind of honey, and our sample reached almost 40°G. Hydroxymethylfurfural, pH and acidity were higher than those observed in other samples of different floral origin. The ratio F/G was 1·21, maltose proportion was 8·09%, and erlose was the only trisaccharide detected (0·74%).

Blueweed honey (*Echium* sp.)

This coarse prickly weed yields up to 500 kg honey/ha (Crane, 1975). Louveaux and Vergeron (1964) mentioned that pollen of *Erica umbellata* L., Cistaceae and Labiatae often appears in blueweed honey. This type of honey is light golden. Moisture was low (16·40%), HMF reached 13·85 mg/kg and diastase activity was 15·75°G. pH was 4·04 and free acidity reached 24·24 meq/kg. The ratio F/G was as high as 1·35, whereas sucrose content was only 0·41%.

Spike lavender and French lavender honeys (*Lavandula latifolia* Med. and *L. stoechas* L.)

This honey is light amber, with typical flavour, and is fast crystallising (Piana *et al.*, 1989). Serra (1988) studied the honey of *L. latifolia* Med. and reported low sucrose proportion, average conductivity, high diastase activity and ash content of 0·15%. Our sample contained 1·09% sucrose, the ratio F/G was 1·18, conductivity was low (1·84 10^{-4}S × cm^{-1}) and ash content only reached 0·06%. HMF was below 1 mg/kg and diastase activity was 25·38°G, a lower value than that reported by Serra (1988) in spike lavender honey.

Honey of *L. stoechas* L. yielded similar results, but HMF was higher (4·79 mg/kg) and diastase activity was lower (10·79°G). pH was 3·74 and free acidity was 14 meq/kg. This sample had high fructose proportion (40·46%), similar to that of 38·20% reported by Serra and Ventura (1993), and the highest ratio F/G (1·41). However, the sucrose proportion was only 0·35% and maltose 6·69%, whereas melezitose was not detected.

Vetch honey (*Vicia sativa*)

The genus *Vicia* includes valuable fodder and soil-building plants as well as a few that are toxic. Vetch honey is amber, strong-flavoured and crystallises in a coarse way. This honey is slightly levorotatory (α = −1·95). pH was 3·94 and diastase activity reached 38·40°G. The ratio F/G was 1·24, the sucrose proportion was 0·67% and melezitose was not detected.

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