Characterization of Citrus Honey (*Citrus* Spp.) Produced in Spain

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The palynological and physicochemical properties of 15 citrus honey samples commercially produced in Spain are reported. Methyl anthranilate contained in citrus honey gives a distinctive flavor to the product. The percentage of sucrose largely exceeds the legal limits established for honey by EU (5 g/100 g) and FAO/WHO (10 g/100 g), which require at least 1–3 months of ripeness to reach legal requirements. The profile sugars and methyl anthranilate change with the transition period before honey commercialization. Fresh spanish citrus honey must be classified with the group of abundant nectariferous flow honeys in order to admit its higher sucrose content.

Keywords: *Citrus honey; methyl anthranilate; sugars*

INTRODUCTION

Today, characterization of flavor and quality control of monofloral honeys, citrus honey among them, is a subject of great interest in apiculture. Although it is possible make a partition between the monofloral honeys, the classification between mono- and polyfloral honey can sometimes be unprecise and ambiguous. It is therefore necessary to obtain global and comparative data about the contribution of several orange varieties on citrus honey distinction. The studies on orange (*Citrus* spp.) honey from València (Spanish east coast) have adduced a large number of analytical data (Peris, 1981; Serra Bonvehí et al., 1987; Serra Bonvehí, 1988). The minimum content of methyl anthranilate (MA) was suggested as a suitable selection parameter to characterize the orange (*Citrus* spp.) honey from the Spanish east coast according to the aging of honey (>0.5 mg/kg) (Serra Bonvehí, 1988). It could be possible for this parameter to be used as a differentiating element between honeys (Steeg and Montag, 1988; Blank et al., 1989; Bouseta et al., 1992). Despite having the highest aromatic content found in fresh honeys, a percentage of sucrose higher than 5 g/100 g disables it from commercialization in EU (BOE, 1986). The three puposes of this work have been as follows: (1) to estimate MA content of several honey samples from different geographic areas in Spain; (2) to study the variation of methyl anthranilate contents and hydroxymethylfurfural (HMF), diastase number, and sugars profiles during the transition period to honey commercialization; and (3) to suggest new limits of sucrose and MA contents for a citrus honey (*Citrus* spp.) quality norm bearing in mind the current European legislation.

MATERIALS AND METHODS

Samples. The study was carried out on 15 citrus honeys from different varieties of lemon tree (*Citrus limon* Burm.), orange tree (*Citrus sinensis* Osbeck, *Citrus aurantium* L.), and mandarin orange tree (*Citrus deliciosa* Ten.) from the Spanish east coast, provided from lots destined for sale on the market. The samples were harvested in April 1992. The samples were preserved at 0–5 °C and analyzed as soon as they arrived in the laboratory. After the aroma (MA) analysis they were then preserved at room temperature. The average storing temperature in the laboratory was 20 °C (15–25 °C). The samples were unpasteurized, although some were heated to facilitate their extraction. Analyses were carried out in triplicate.

Table 1. Honey Samples: Site of Origin

sample no.	geogr. origin	% *Citrus* sp. pollen	pollen richness groups[a]
1	València	18	I
2	Córdoba	21	I
3	Córdoba	19	I
4	Castelló	17	I
5	València	18	I
6	València	8	I
7	Castelló	22	I
8	València	10	I
9	Castelló	18	I
10	Castelló	23	I
11	València	18	I
12	València	22	I
13	Castelló	25	I
14	València	17	I
15	Alacant	9	I

[a] I: (PK) pollen counted grains/10 g honey (<20.000).

Melissopalynological Analyses. The analysis was carried out in accordance with the methods of the International Commission of Bee Botany (ICBB) of the International Union of Biological Sciences (IUBS), described by Louveaux et al. (1978). The grain count was performed following the method suggested by Vergeron (1964) (1200 grains counted). The pollens identified were classified according to their frequency. There were four classes of pollen: predominant (>45%) = D; secondary (16–45%) = S; important minor (3–15%) = s; and minor (<3%) = r. For the absolute number of pollen grains, there are five groups: group I = honeys low in pollen [PK (pollen counted/10 g <20 000); group II = normal honeys (PK/10 g 20 000–100 000); group III = honeys rich in pollen (PK/10 g 100 000–500 000); group IV = honeys extremely rich in pollen (PK/10 g 500 000–1 000 000); group V = pressed honeys (PK/10 g >1 000 000).

Physicochemical Analyses. The sensory analysis was carried out under the conditions of the International Standard (ISO 6658, 1985; Serra Bonvehí and Gómez Pajuelo, 1988). The acceptability was tested using a category ordinal scale (1= very bad; 2 = rather bad; 3 = below average; 4 = average; 5 = rather good; 6 = very good; 7 = excellent).

Water content was determined following Chataway (1932) and Wedmore (1955), a method established by the Codex Alimentarius Commission (1969). We used an Atago Model 8326 Abbe-type refractometer.

The color was classified by the Pfund Classifier (Brice et al., 1956). Samples were "read" with a standard froted incandescent bulb 30 cm from the back of the classifier. All readings were taken by one person, except when comparisons

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Table 2. Pollen Spectra[a]

	sample no.														
plant origin	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Aceraceae															
Acer sp.	r					r									
Boraginaceae															
Borago officinalis				r					r				r		
Echium sp.		r			r										r
Caprifoliaceae															
Lonicera sp.			r	r			r				r			r	r
Caryophyllaceae (NN)															
t. Silene		r					r			s		r			
Ciperaceae (NN)															
Carex sp.						r		r					r		
Cistaceae (NN)															
Cistus sp.	s	r	S	r	s	s	r	S	r	r	s	s	r	s	S
Helianthemum sp.		r	s		s			s			s		s		
Compositae															
t. Aster							r								
t. Centaurea			r											r	r
t. Carduus				r											
t. Solidago						r									r
Taraxacum officinale	r	r	r	r	r	s	r	s	r	r	r	r	r	r	s
Coniferae (NN)															
Pinus sp.									r		r				
Cruciferae															
t. Raphanus	r	r		r		r			r						
t. Sinapis			r		r							r			
t. Diplotaxis	S	s	s	s	S	S	s	S	s	S	s	s	s	S	S
Ericaceae															
Erica sp.		r		r			r								
Fagaceae (NN)															
Quercus sp.	S	s	s	S	s	S	s	s	S	S	s	S	s	S	S
Gramineae (NN)															
t. 30-60 μ		r				r			r						
Labiatae															
Lavandula sp.	r		r		r			r							
Rosmarinus officinalis		r				r	r			r	r	r	r		
Liliaceae															
t. Asphodelus				r			r								
Leguminosae															
Anthyllis cytisoides						r								r	r
t. Dorycnium	r	r			r					r				r	
t. Genista				r				r				r			
Lotus corniculasus			r				r				r			r	r
Onobrychis viciifolia					r							r	r		
Trifolium repens		r				r			r	r					
Myrtaceae															
Myrtus comunis						r	r				r			r	
Oleaceae															
t. Phillyrea	r			r		r		r		r					r
t. Ligustrum			r						r				r		
Olea europeae		r	r		r		r		r		r	r		r	
Oxalidaceae															
Oxalis sp.				r		r			r	r					r
Papaveraceae															
Hypecoum sp.	r		r		r		r	r		r	r		r	r	r
Plantaginaceae (NN)															
Plantago sp.	r	r	r	r	r			r			r	r		r	r
Ranunculaceae															
t. Ranunculus							r			r					
Rhamnaceae															
Rhamnus alaternus	r			r				r							
Rosaceae															
Crataegus monogyma												r			r
t. Prunus		r			r	r			r						
t. Pyrus				r							r		r	r	
Rubus sp.							r			r			r		
Rubiaceae															
t. Gallium	r		r					r				r			
Rutaceae															
Citrus sp.	S	S	S	S	S	s	S	s	S	S	S	S	S	S	s
Salicaceae															
Populus sp.	r						r			r			r		

Table 2 (Continued)

plant origin	sample no. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Salix sp.	r	r	r	s	r	r	r	r	s	r	s	s	r	r	r
Ulmaceae															
t. Ulmus									r						r
Umbelliferae															
t. Eryngium						r					r				
Vitaceae															
Vitis vinifera	r						r			r			r	r	

[a] Key: D, predominant pollen (>45%); S, secondary pollen (16–45%); s, important minor pollen (3–15%); r, minor pollen (<3%); t, type; NN, nectarless plant.

Table 3. Physicochemical Characterization and Composition[a]

parameters	sample no. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	x	SD
water content	18.7	18.6	17.1	16.8	16.5	17.3	16.3	16.1	16.9	16.9	17.8	16.7	16.1	17.2	18.6	17.2	0.88
color	6	12	12	7	17	14	11	11	11	3	12	21	4	17	18	11.70	5.20
HMF	0.2	0.15	0.9	1.3	0.5	1.1	0.6	0.1	0.1	0.7	0.3	0.6	0.3	0.7	1.1	0.58	0.39
diastase no.	14.6	17.0	18.1	20.0	13.0	14.9	16.3	17.4	15.1	19.0	17.9	15.9	22.3	21.0	20.9	17.60	2.69
MA	2.00	3.60	3.26	2.46	2.34	2.44	2.66	2.74	2.35	2.32	2.83	2.03	1.78	2.49	2.35	2.51	0.47
fructose	32.2	34.6	34.0	33.7	33.0	33.0	34.2	33.2	33.8	33.2	30.3	34.9	31.6	33.6	34.4	33.3	1.22
glucose	27.3	29.4	28.2	28.4	27.7	27.9	28.5	28.5	28.6	27.9	25.6	28.8	26.4	28.3	28.7	28.0	0.97
sucrose	16.3	13.5	11.8	13.6	14.6	16.3	9.60	15.6	12.4	15.2	15.5	10.2	15.0	12.4	9.4	13.4	2.37
trehalose	0.20	0.20	0.25	0.25	0.26	0.24	0.20	0.22	0.24	0.22	0.13	0.10	0.23	0.30	0.22	0.22	0. 05
isomaltose	0.23	0.24	0.37	0.48	0.41	0.38	0.51	0.35	0.36	0.28	0.26	0.68	0.37	0.49	0.39	0.39	0.12
maltose	2.51	2.95	2.95	3.87	3.85	3.30	4.33	3.21	3.49	2.80	2.78	4.71	3.15	3.73	3.22	3.40	0.60
melezitose	tr	tr	tr	tr	tr	tr	0.10	tr	tr	0.11	tr	0.10	tr	tr	tr	tr	
raffinose	tr	tr	0.19	tr	tr	tr	tr	tr	0.31	tr	0.26	tr	tr	0.22	tr	tr	
erlose	tr	tr	tr	tr	0.38	tr	tr	tr	0.19	tr	0.36	tr	tr	tr	0.19	tr	
sugar content	78.4	80.9	78.5	80.4	80.3	81.2	77.6	81.1	79.7	79.7	75.3	79.3	76.8	79.3	76.5	79.0	1.79
sensory analysis	4	6	6	5	6	6	6	5	6	6	6	5	4	5	6	5.5	0.74

[a] Key: water content, g/100 g; color, mm Pfund scale; HMF ((hydroxymethyl)furfural), mg/kg; diastase number, Gothe's scale; MA (methyl anthranilate), mg/kg; sugars, g/100 g.

between readers were made. Each reading recorded was the average of five single readings by the same person.

Carbohydrates were determined based on the analyses of their oxime trimethylsilyl derivatives by the gas chromatography method described by Serra Bonvehí and Bosch Callís (1989) on a GR-6A gas chromatograph and quantified on a CR-3A (Shimadzu) microprocessor.

Diastase Number. The procedure of Schade et al. (1958), modified by White (1964), was used. The diastase induced hydrolysis in a starch solution (Merck Art. 1252), which fulfills the requeriments of the method [AOAC (31.162–31.167), 1984; BOE, 1986]. Solutions were thermostated in a Unitronic 320 OR (Selecta, Barcelona) ultrathermostatic bath. Absorption was followed using a Hitachi U-2000 UV–vis double-beam spectrophotometer. Using regression, lines were fitted to the absorption data and the diastase number was calculated from the time taken for the absorbance to reach 0.235.

Methyl Anthranilate (MA). The MA was determined by the technique originally described by Serra Bonvehí (1988). A 10 g honey sample was weighed and extracted three times with 20 mL portions of methylene chloride. The organic extracts were mixed and evaporated at 30 °C in a rotavapor to 1 mL. The MA was then detected and identified on a Sigma 2 gas chromatograph with a flame ionization detector (GC-FID) and quantified on a Sigma 15 (Perkin-Elmer) microprocessor under the following conditions: 2 m glass column (3 mm o.d.) packed with 1.5% SP-1000 on Chromosorb G 60/80 mesh, carrier gas 25 mL of N_2/min; FID with H_2 at 30 mL/min and O_2 at 400 mL/min. The temperatures wereas follows: injector, 250 °C; detector, 260 °C; column, 180 °C; rate 6 °C/min to 220 °C, held for 5 min. Internal standard calibration was performed with eicosane. Methyl anthranilate and eicosane were obtained from Sigma (St. Louis, MO).

(Hydroxymethyl)furfural (HMF). Determination made according to White's (1979) method. Honey samples were divided into two clarified aliquots. Water was added to one, and the ultraviolet absorption in the solution due to the presence of HMF was then read against that of a solution in which this absorption was previously destroyed by the addition of sodium bisulfite solution to break the double conjugated band responsible [AOAC (31.152–31.155)], 1984; BOE, 1986]. A Hitachi U-2000 UV–vis double-beam spectrophotometer was used.

RESULTS AND DISCUSSION

The high variability of the percentage of *Citrus* sp. pollen makes it difficult establish a minimum percentage to qualify orange honey (Table 1). All samples belonged to group I. In the 15 samples different pollen types were identified with an average of 17 per sample. Table 2 shows data from the pollen analysis, identifying the following in the majority of samples: *Cistus* sp., *Brassicaceae*, *Plantago* sp., *Quercus* sp., *Taraxacum officinale*, and *Salix* sp. *Hypecoum* sp. pollen, considered as the marker of Spanish honeys, was present in approximately 66% of the samples, in agreement with Sala Llinares (1986). Also, the presence of *Olea europaea* and *Quercus* sp. was remarkable, according to Weber (1982), Serra Bonvehí et al. (1987), and Munuera and Carrión (1994). Table 3 gives values of physical and chemical parameters for the characterization and sensory assessment. Important sucrose percentages were detected, surpassing the maximum admited value in the EU (5 g/100 g) (BOE, 1986). Only the honey that proceeds from abundant nectariferous flow plants (e.g., *Lavandula spica* × *latifolia*, *Robinia pseudoacacia* L.) may contain greater sucrose quantities (10 g/100 g honey) and lower diastase activity (3 Gothe's scale). Erlose, raffinose, and melezitose were identified in some samples in agreement with the finding of Battaglini and Bosi (1973), Peris (1981), and Serra Bonvehí et al. (1987). The evolution of sugars during the first 3 months after collection of honey was determined in samples 1, 4, 6, and 12. An important loss of sucrose

Table 4. Sugars Profile, MA, HMF, and Diastase Number Evolution[a]

	sample 1			sample 4			sample 6			sample 12		
parameters	1	2	3	1	2	3	1	2	3	1	2	3
fructose	32.2	34.9	36.3	33.7	36.3	37.2	33.0	34.7	36.9	34.9	34.4	38.3
glucose	27.2	29.7	30.3	28.3	30.3	29.7	27.9	29.4	29.8	28.80	27.80	30.3
sucrose	16.3	8.80	5.90	13.6	6.40	2.60	16.3	9.10	4.50	10.20	5.00	2.70
trehalose	0.20	0.17	0.41	0.25	0.42	0.53	0.24	0.36	0.43	0.10	0.43	0.61
isomaltose	0.23	0.45	0.72	0.48	1.06	1.43	0.38	0.76	1.07	0.68	0.96	1.42
maltose	2.51	3.95	4.49	3.87	5.71	6.74	3.30	4.67	5.54	4.71	5.74	6.92
gentibiose	tr	0.10	0.11	tr	0.20	0.26	tr	0.10	0.18	tr	0.19	0.26
melibiose	tr	0.10	0.10	0.10	0.16	0.26	tr	0.10	0.14	tr	0.17	0.21
sugar content	78.7	78.2	78.4	80.4	80.6	78.8	81.2	79.2	78.6	79.3	74.8	80.9
I.D.	14.6	12.6	11.2	20.0	18.2	15.5	14.9	13.5	10.3	15.9	16.3	13.1
MA	2.00	1.87	1.70	2.46	2.30	2.16	2.44	2.27	2.10	2.03	1.83	1.68
HMF	0.30	0.60	1.10	1.30	1.90	2.50	1.10	1.70	2.10	0.60	1.30	2.40

[a] Key: 1, 2, 3, months in storage; sugars, g/100 g; I. D., diastase number (Gothe's scale); MA, methyl anthranilate (mg/kg); HMF, (hydroxymethyl)furfural (mg/kg).

content, between 63% and 81% of the initial value, was observed, while fructose, glucose, trehalose, isomaltose, and maltose contents increased according to Echigo and Takenaka (1973) and Takenaka and Echigo (1976, 1978). Fresh Spanish citrus honey cannot be commercialized because its initial sucrose content largely exceeds the limit established by legal requirement (BOE, 1986). For sample 1, the sucrose content was only reduced to inferior values of 5 g/100 g after 3 months (Table 4). These variations in the sugar spectrum were also found in citrus honey (1986 harvest) (Serra Bonvehí et al., 1987). Of the sucrose present an average of 48% is lost per month. Three months of storage is probably sufficient to meet the European legislation of 5% and 1 month is sufficient for the recommendations of the Codex Alimentarius Commission (1988) (10 g/100 g), with a consequent lesser loss of MA. Methyl anthranilate loss is consistent at about 9% of that present per month. Excluding sample 13, all samples had a minimum content of 2.00 mg/kg of methyl anthranilate, varying from 1.78 to 3.60 mg/kg ($x = 2.51 \pm 0.47$) (Table 3). This presence fundamentally depends on cultivated citrus varieties, water content, degree of freshness, and apiarian exploitation method. The diastatic activity and the (hydroxymethyl)-furfural (HMF) content are widely recognized parameters in evaluating the freshness of honey (Schade et al., 1958; Sancho et al., 1992). Legal regulations in the European Union establish a minimum value for diastase activity of eight on Gothe's scale and a maximum HMF content of 40 mg/kg. In honeys with low enzymatic content a diastase number of three on Gothe's scale is permisible as long as HMF content does not exceed 15 mg/kg (citrus honey). Talpay (1987) fixed at least 2 mg/kg of methyl anthranilate on citrus honey. This limit agrees with the sensory result (White, 1966). In the case of aging honeys (HMF content >10 mg/kg) this value is reduced to 0.50 mg/kg to be classified as monofloral for spanish varieties (Serra Bonvehí, 1988). In addition to the loss of sucrose hydrolysis, the aromatic content of spanish citrus honey (*Citrus limon* Burm.) also decreases (1.5–2 mg/kg), with the consequent difficulty of commercialization (Serra Bonvehí, 1988). Honey samples showed an appropiate diastase number ranging from 13 to 22.3°G, and their HMF content averaged 0.58 mg/kg with a maximum of 1.30 (Table 3). Diastase number is considered normal, in agreement with Peris (1981) and Serra Bonvehí et al. (1987). A low diastase number in citrus honey is admitted by European regulation, but the results found did not correspond ($x = 17.60 \pm 2.69$ Gothe's scale). The diastase values of the same samples (samples numbers 1, 4, 6, and 12) in the first 3 months after collection were analyzed (Table 4). A loss of enzymatic activity (between 17.61% and 30.87%) was found, representing an average variation of 1.27 Gothe units/month at room temperature, reducing the analytical quality of honey according to Sancho et al. (1992). Typical honey from flowerings with very abundant nectariferous flow must be extracted before its total ripeness, owing to the deficiency of available room in Layens hive of 12 frames used in Spain. Early extraction of honey also contributes in the reduction its aromatic content. (Hydroxymethyl)furfural values were suitable for a well preserved fresh honey, but increasing from transition period to honey commercialization (Serra Bonvehí, 1991). In keeping with these results and with sensory analysis, all the samples just collected can be evaluated as orange (*Citrus* sp.) honey and have the sucrose content of <10 g/100 g and MA content of >1.5 mg/kg, a characteristic of Spanish citrus honey.

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