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Sugar profiles of Spanish unifloral honeys

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The levels of various sugars (fructose, glucose, sucrose, maltose, maltulose, kojibiose, isomaltose, raffinose, erlose and melezitose) as well as the glucose/fructose and glucose/water ratios were determined in different Spanish unifloral honey types (rosemary, orange blossom, lavender, sunflower, eucalyptus, heather, honeydew). Sugars were determined by gas chromatography of the trimethylsilyloxime derivatives. There were significant differences among the honey types in relation to sugar composition. Fructose, glucose, sucrose, maltose and the glucose/water ratio were selected by discriminant analysis as the better parameters for the correct classification of the honey samples into their parent types. These sugars appear to be very valuable as characterization parameters for honeydew honey, followed by sunflower, heather and eucalyptus honeys, with 100%, 92.9%, 83.3% and 75.0% correct classifications, respectively. For the remaining honey types the percentages of successful classifications ranged from 53.8% to 69.2%. © 1997 Elsevier Science Ltd

INTRODUCTION

Honey consumers are generally worried by quality and have frequently demanded an origin denomination which would guarantee that quality. Most consumers have demanded not only a basic quality level but a clear certificate of geographical and botanical origin. This has occurred in some European countries and has led to regulations in different countries. In Spain, the honey regulation (Anonymous, 1983) states that geographical and botanical origin of this product must be shown on package labels. Control of honey requires the determination of parameters that could unequivocally establish origin and calls for efforts to improve honey characterization.

Identification and count of pollen and other honey microscopic components such as algal cells and fungal spores have been used for authentication. Honey microscopy was included in directives for carrying out honey control orders in Germany and Switzerland, although there are difficulties for a correct assignment of the origin (Maurizio, 1975*a*). Some physicochemical parameters such as electrical conductivity (Vorwohl, 1964), pH, sugars, α-amylase activity or thixotropy are considered useful, whereas pollen analysis should to be kept as a secondary tool for confirming the origin established by physicochemical measurements (Pourtallier & Taliercio, 1970). The profile obtained by gas chromatography (GC) of sugar trimethylsilyl (TMS) ethers was judged to be a basic characterization, but critical values were established only for a few sugars and some unifloral honey types (Pourtallier, 1967, 1968; Pourtallier & Taliercio, 1970; Institut Technique d'Apiculture, 1975).

Battaglini and Bosi (1972, 1973) studied this topic by GC, concluding that honey sugars are related to those present in the raw materials (nectar or honeydew) foraged by bees to make a unifloral honey in such a way that identification of the source could be possible. Grandi (1977), using charcoal column, thin-layer and gas chromatography, found qualitative differences among the chromatograms of five Italian unifloral honey types, but only relating to peaks that he was unable to identify.

Sugars contained in nectars are mainly fructose, glucose and sucrose, but their relative proportions are usually rather variable; however, they are quite consistent for certain botanical families (Maurizio, 1975*b*; Baker & Baker, 1983). These last authors consider four nectar classes as a function of the sucrose/hexose (S/H) ratio: 'sucrose dominant' ($S/H > 0.999$), 'sucrose rich' ($0.5 < 0.999$), 'hexose rich' ($0.1 < 0.499$) and 'hexose dominant' ($S/H < 0.1$). Other sugars such as maltose, melibiose, gentiobiose or the trisaccharides raffinose and melezitose are rare (Percival, 1961; Pais & Chaves das Neves, 1980). Honeydew is secreted by some species of plant-suckling insects and falls on to the surface of leaves, fruits and twigs (Maurizio, 1975*b*). Besides fructose, glucose and sucrose, some oligosaccharides, such as maltose, α,α-trehalose, erlose, raffinose and

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melezitose, have been reported to be found in honeydew (Maurizio, 1975*a*; Lombard *et al.*, 1984).

The great variety of sugars (especially di- and trisaccharides) found in honey (Siddiqui & Furgala, 1970), in contrast with the simplicity of their sources, has been related to changes undergone by raw sugars due to α-glucosidase originating in honeybees. The role played by other enzymes such as β-glucosidase (Low *et al.*, 1986), or α- and β-amylases is not clear.

Some work has been developed in order to examine the sugars of various honey types from certain areas of Spain (Serra *et al.*, 1987), but more extensive effort is necessary.

The aim of this work was to study (by GC) the sugar composition of the most important Spanish unifloral honey types and to evaluate the suitability of the GC sugar spectrum as a practical way to characterize these honeys. The classes of honeys examined are: rosemary, orange blossom, lavender, sunflower, eucalyptus, heather and honeydew.

MATERIALS AND METHODS

Apparatus

An Abbé 60 refractometer, standard model (Bellingham & Stanley, UK) was connected to a Termotronic S-389 thermostatic bath (Selecta, Barcelona, Spain).

A Sigma 3 gas chromatograph (Perkin-Elmer, Norwalk, CN, USA) was equipped with two flame-ionization detectors and two packed columns—stainless-steel, 3 m × $\frac{1}{8}$ inch o.d., 3% OV-17 on 80–100 mesh Chromosorb W(HP)—connected to a HP 3390A integrator (Hewlett Packard, Avondale, PA, USA).

A heating-stirring module was used for reacti-vials (Pierce Chemical Co., Rockford, IL, USA).

Reagents and standards

1,1,1,3,3,3-Hexamethyldisilazane (HMDS), hydroxylamine hydrochloride, n-octadecane (n-C_{18}), and trifluoroacetic acid (TFA) were purchased from Merck (Darmstadt, FRG). Anhydrous pyridine was a product of Scharlau (Barcelona, Spain). Anhydrous magnesium sulphate was purchased from Probus (Barcelona, Spain). Triphenylethylene 'purum' was obtained from Fluka (Buchs, Switzerland).

The following sugar standards were used: anhydrous glucose and fructose, sucrose, maltose monohydrate, α,α-trehalose, melibiose monohydrate, raffinose pentahydrate (Merck), turanose and isomaltose (Sigma Chemical Co., St Louis, MO, USA), gentiobiose and melezitose dihydrate (Fluka), kojibiose (Koch-Light, Colnbrook, UK), maltulose monohydrate (a gift from Dr H. D. Scobell, A. E. Staley Mfg., Decatur, IL, USA), nigerose (provided by Prof. P. J. Reilly, Univiversity of Ames, IA, USA) and erlose (donated by Prof. S. Chiba, University of Sapporo, Japan).

An oxime–internal standard solution was prepared by dissolving hydroxylamine hydrochloride (50 mg ml^{-1}), n-C_{18} (8 mg ml^{-1}) and triphenylethylene (3.5 mg ml^{-1}) in anhydrous pyridine.

Samples

Reportedly unifloral honey samples were obtained from beekeepers, dealers or official services. They were produced in different Spanish regions and were from different year crops; while some of them were crude, others had been subjected to some heating. In any case, they were analyzed as soon as received in the laboratory. Their botanical origin was first ascertained by palynological analysis according to the International Commission for Bee Botany (Louveaux *et al.*, 1978), together with sensory assessment of their colour, flavour and aroma. Pollen analysis was not applicable to honeydew honey, which necessitated determining its electrical conductivity (> 800 μS cm^{-1}) and pH (> 4.3) (Vorwohl, 1964; Bosch & Mateo, 1984). The honey samples used were as follows: 13 from rosemary (*Rosmarinus officinalis* L.), 16 from orange blossom (*Citrus* spp.), 15 from lavender (*Lavandula latifolia* Med.), 14 from sunflower (*Helianthus annuus* L.), 14 from eucalyptus (*Eucalyptus camaldulensis* Dehnh. and *Eucalyptus globulus* Labill.), 13 from heather (Ericaceae) and 16 from honeydew honey (*Quercus* spp.). The samples were homogenized by mild warming (at a temperature below 50°C) and shaking, and then filtered through 0.25 mm mesh sieves.

Moisture

Moisture was determined by measuring the refractive index at 20°C in an Abbé refractometer, according to the official methods of analysis of the AOAC (1980).

Sugar analysis

Sugars were determined by GC as their trimethylsilyloxime derivatives following the technique of Pourtallier and Rognone (1977) further modified by us (Mateo & Bosch, 1984).

Derivatization

Sugar standards were dissolved in anhydrous pyridine at the following concentrations: 10–18 mg ml^{-1} for glucose and fructose; 0.5–5 mg ml^{-1} for di- and trisaccharides. Then 2.5 ml of each solution were mixed in a tube with 2.5 ml of the oxime–internal standard solution; n-C_{18} was used as internal standard and reference compound and triphenylethylene was employed as a second reference compound. Anhydrous magnesium sulphate (2 g) was added as desiccant. The tube was closed with a PTFE-lined screw cap, shaken and kept in a horizontal position for about 16 h (usually overnight). After the solid phase had settled, 1.0 ml of the supernatant solution was transferred to a reacti-vial (Pierce Chemical Co.) and mixed with 1.0 ml of HMDS and 0.1 ml of

TFA. The vial was capped with a Mininert valve and heated at 80°C for 1 h in a heating-stirring module. The vial was cooled and, after the white precipitate had settled, 3 µl of the supernatant solution were injected into the gas chromatograph. Calibration curves were obtained for each sugar and used for quantification. More dilute solutions were prepared when needed in order to determine the limits of detection.

Liquid honey samples were dissolved in anhydrous pyridine (4 g per 100 ml solution). The solutions (2.5 ml) were mixed with the same volume of the oxime-internal standard solution and treated as described above for standards. Each sample was injected 5–6 times.

Chromatographic conditions

Injector and detectors were set at 280°C. The oven temperature was programmed from 175°C to 280°C at 2°C min^{-1} and held at 280°C for 10 min. Helium was used as carrier gas at a flow-rate of 30 ml min^{-1}.

Statistics

The concentrations of sugars in honeys as well as the fructose/glucose (F/G) and glucose/water (G/W) ratios were statistically tested. We used monovariate analysis (analysis of variance, Tukey's test, Kolmogorov–Smirnov test of good fitness) from the SPSS package (SPSS, 1986) and multivariate analysis (stepwise discriminant analysis, BMDP7M) from the BMDP statistic package (Dixon *et al.*, 1985). Calculations were performed on a Honeywell Bull computer.

RESULTS AND DISCUSSION

Sugar identification

Table 1 lists the number of peaks obtained for each sugar under the working conditions and their area ratios; an arbitrary value of 10 was attributed to the main peak according to Toba and Adachi (1977). The retention times (RRT) of sugars relative to time of n-C_{18} and triphenylethylene are listed; they were usually used for identification of the chromatographic peaks eluted during the analysis of samples. Middle- and last-eluted sugars were identified more accurately with triphenylethylene as reference because of the better precision of their RRTs.

Fructose and glucose each yielded only one peak as the syn- and anti-isomers are not separated on the OV-17

Table 1. Number of chromatographic peaks, relative rentention times and peak area ratios of the oxime trimethylsilyl ethers of some sugars related to honey

Sugar	Peak number	Peak area ratio[c]	Retention time relative to:			
			n-Octadecane[a]		Triphenylethylene[b]	
			Mean	RSD (%)	Mean	RSD (%)
Fructose	1	—	1.304	0.38	0.288	0.76
Glucose	1	—	1.576	0.47	0.348	0.60
Sucrose[d]	1	—	5.25	1.05	1.159	0.13
Trehalose[d]	1	—	5.86	1.08	1.294	0.15
Maltulose	1	9	6.09	1.06	1.331	0.16
	2	10	6.17	1.06	1.348	0.16
Maltose	1	—	6.28	1.08	1.387	0.17
Nigerose	1	10	6.27	1.15	1.368	0.18
	2	3	6.61	0.98	1.442	0.19
Turanose	1	—	6.31	0.87	1.389	0.14
Kojibiose	1	10	6.50	0.78	1.418	0.10
	2	2	6.71	0.72	1.463	0.11
Palatinose	1	10	6.76	0.80	1.475	0.14
	2	10	6.87	0.82	1.500	0.15
Gentiobiose	1	1.5	6.71	0.67	1.465	0.11
	2	10	6.93	0.74	1.514	0.13
	3	3	7.06	0.76	1.543	0.17
Melibiose	1	2	6.83	0.92	1.509	0.21
	2	10	6.97	0.95	1.548	0.21
	3	3	7.16	0.98	1.590	0.24
Isomaltose	1	2	6.98	1.3	1.525	0.36
	2	10	7.18	1.3	1.570	0.39
	3	3	7.36	1.3	1.609	0.43
Raffinose[d]	1	—	9.5	1.6	2.07	0.63
Melezitose[d]	1	—	9.9	1.8	2.16	0.70

For chromatographic conditions: see text.
[a]Retention time of n-octadecane (mean ± SD): 5.87 ± 0.12 min.
[b]Retention time of triphenylethylene (mean ± SD): 26.9 ± 0.32 min.
[c]A value of 10 is assigned to the main peak.
[d]Non-reducing sugar that yields the trimethylsilyl ether.

packed column, according Petersson (1974), Zürcher *et al.* (1975) and Demaimay (1977). The non-reducing sugars, sucrose, α,α-trehalose, raffinose and melezitose, each yielded single peaks corresponding to their TMS ethers. Maltose gave one peak that overlapped the main peak of nigerose. Melibiose, isomaltose and gentiobiose each provided three peaks, the second of them being the more important. Among the ketodisaccharides, maltulose and palatinose yielded two partially overlapping peaks with slightly different areas, but turanose yielded only one peak since the two isomers were not resolved and overlapped with maltose. Our results agree well with those of Toba and Adachi (1977), except for gentiobiose and melibiose. They found different area ratios for the peaks of these sugars but they also detected other minor peaks, which were suggested to be related to two isomeric cyclic modifications of oximes. Differences may arise both from the different derivatization treatment and the chromatographic conditions. Relative variability of peak area ratios suggests that by-products from partial degradation in the chromatographic system may cause the presence of more than two peaks.

Some peaks from these sugars overlap peaks from other sugars, which complicates both qualitative and quantitative analysis.

When applied to unifloral honeys previously accepted on the basis of pollen analysis and organoleptic properties, differences in chromatographic profiles were found. Figure 1 shows the chromatogram of a honeydew honey. The following sugars were usually identified: fructose, glucose, sucrose (sometimes at very low levels), maltulose, maltose (overlapping with nigerose and turanose), kojibiose, isomaltose, raffinose and melezitose. Trisaccharides were not usually detected in sunflower and heather honeys. Small unidentified peaks appearing between sucrose and maltulose were generally noticeable. The RRT of one of them was slightly lower than that of the α,α-trehalose standard. The peak of this disaccharide, although observed in some samples of honeydew honey, was not detected in floral honey samples, in agreement with Siddiqui and Furgala (1967). Melibiose was not detected in any samples, which agrees with some authors (Siddiqui & Furgala, 1967; Battaglini & Bosi, 1973; Hadorn *et al.*, 1974; Grandi, 1977; Swallow & Low, 1990), but not with others (Pourtallier, 1968; Serra *et al.*, 1987).

Among the trisaccharides, two peaks, attributed to raffinose and melezitose on the basis of their RRTs, were observed in many samples. A peak appearing just in front of the melezitose peak was noticeable in many samples. It was attributed to erlose, a trisaccharide often reported in honey. This peak disappeared when honey was hydrolysed with β-D-fructosidase from yeasts (Boehringer Mannheim, FRG) following the method of Lombard *et al.* (1984); at the time, the peaks from fructose and maltose increased slightly, which is consistent with this hypothesis. Further availability of erlose standard, when the bulk experimental work had finished, confirmed the supposition.

Quantitative analysis

Sugars were determined using n-C_{18} as internal standard. Because of difficulties with resolution of disaccharides and trisaccharides (or unavailability of standards), the following sugars were generally quantified: fructose, glucose, sucrose, 'maltose', maltulose, kojibiose, isomaltose, raffinose, melezitose and erlose.

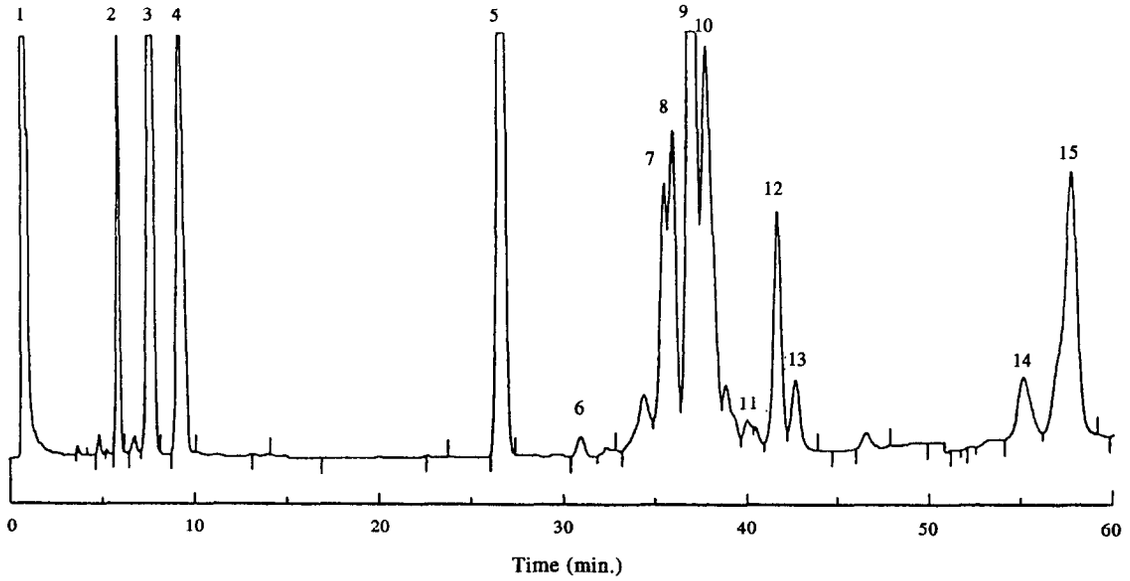

Fig. 1. Gas chromatogram of the reducing sugar trimethylsilyl oximes and non-reducing sugar trimethylsilyl ethers of a honeydew honey (*Quercus* spp.). Peaks are identified as: 1, solvent; 2, n-octadecane (internal standard); 3, fructose; 4, glucose; 5, triphenylethylene (reference); 6, sucrose; 7 and 8, maltulose; 9, maltose, turanose and nigerose; 10, kojibiose; 11, 12 and 13, isomaltose; 14, raffinose; 15, melezitose showing a frontal shoulder of erlose. Chromatographic conditions: see text.

Only a few milligrams of maltulose standard became available, so we used a response factor instead of a calibration curve. The 'maltose' term accounts for the sum of any maltose, nigerose and turanose together and hence it is an overestimation of true maltose. Erlose was estimated using the calibration curve of melezitose because of unavailability of the standard at the time of the experimental work. When it became available the experimental labour had finished; however, we could confirm that the calculation procedure was valid. The F/G and G/W ratios were also calculated and evaluated as possible indicators of botanical origin.

Table 2 shows the means, standard deviations and ranges of the data obtained from the analysis of the different honey types under study.

The Kolmogorov–Smirnov test does not allow rejection of the hypothesis of normality of the distributions ($P > 0.95$). The analysis of variance shows very significant differences ($P > 0.99$) among the values of the sugar parameters in relation to honey type. Tukey's test ($P = 0.95$) arranges honeys in a variety of patterns depending on the sugar under consideration. Honeys grouped together are not differentiated by means of the parameter used for testing. Statistically, and considering the parameter tested, a honey type included in two groups cannot be considered different from others included in both groups. Trisaccharides were excluded because they were not detected or not accurately determined (RSD = 10–15%) in many samples.

The honey groups obtained by Tukey's test can be seen in Fig. 2. Left and right limits for each group are, respectively, the lower and the higher 95% confidence limits for the means of the honey types included in each group.

The ranges for fructose contents overlapped among the honey types studied, although honeydew honeys showed low concentrations of this disaccharide (mean = 34.3%). Tukey's test gives a sole group for this class of honey (Fig. 2). Fructose levels were high in eucalyptus, lavender and especially sunflower honeys (mean = 39.4%). Orange blossom, rosemary and heather honeys showed intermediate fructose levels.

In the case of glucose, however, the separations are not so clear due to major variability for glucose data among all honey types (Table 2). The highest variability was shown by rosemary, orange blossom and lavender honeys (SD about ±2.3%). Three well-separated groups are obtained for glucose content according to Tukey's test: honeydew honey (25.8 ± 1.5%), sunflower honey (35.4 ± 1.4%) and the remaining honey types (at an intermediate situation) (Fig. 2). Sunflower honeys showed the greatest contents of both hexoses, which agrees with sugar composition of Asteraceae nectars (Baker & Baker, 1983). On the other hand, *Quercus* honeydew honeys were poor in both hexoses, as reported for honeydew honey by other authors (White *et al.*, 1962).

The F/G ratio divides the honey population studied into four Tukey's groups, although each overlaps with at least two other groups because of the broad distribution of this parameter. In this way, honeydew and heather honeys, with the higher F/G ratios, form a group that does not overlap with the group of sunflower and rosemary honeys, with lower F/G ratios. This parameter had a broad distribution among rosemary honeys (SD = ±0.1). The great variability of this ratio is imputed more to changes in glucose than in fructose percentages. The F/G ratios for Spanish orange and eucalyptus honeys were higher than the values reported for the Italian ones (Battaglini & Bosi, 1973), but are in agreement with the data from Petrov for Australian eucalyptus honeys (Petrov, 1972).

The G/W ratio gives rise to three groups according to Tukey's test (Fig. 2). The group of eucalyptus and sunflower honeys shows values that are significantly higher than the values from the other honey types. Lavender honeys form a single group, with intermediate G/W ratios. The high G/W ratios of Spanish sunflower honeys correlate well with their high glucose levels and their low moisture, as they are summer honeys, gathered and harvested in hot dry areas of Spain. The high G/W ratio of eucalyptus honeys is due mainly to their low water content.

The distribution of sucrose was very variable in orange honeys. It averaged 4.45 ± 3.3%, being significantly different from the other honey varieties under study. The range of sucrose in *Citrus* honeys overlapped with only part of the ranges of rosemary and lavender honeys. Tukey's test divides the total honey population into two groups: one composed of orange blossom honeys, the other of the remaining honey types. The relatively high sucrose levels found in rosemary and lavender honeys agree with the predominance of sucrose over hexoses in the nectar of Lamiaceae (Percival, 1961; Baker & Baker, 1983). The highest limit for 'apparent sucrose' in Spanish honey regulations is 5% (Anonymous, 1983). It was surpassed by five orange blossom and two rosemary honeys. These high values were usually associated with a high moisture level and may be related to lack of honey ripening. The grounds for this event, in the case of *Citrus* honey, are the concern of beekeepers in Eastern Spain both for exploiting the exuberant orange blossom flowering and for saving their bees from the hazards of agricultural pesticides. The lowest sucrose levels were found in sunflower and heather honeys where the concentration did not exceed 0.15% and 0.20%, respectively.

As can be seen from Table 2, the distribution of 'maltose' levels in the honey types studied was broad, except for sunflower honeys, which showed the lowest percentages (2.7% ± 0.3%). Four groups can be depicted from Tukey's test, but they overlap in the same way as the groups found for the F/G ratio (Fig. 2). The utility of this parameter seems to be the differentiation between sunflower and eucalyptus or honeydew honeys, which are rich in maltose. *Citrus* honeys are relatively poor in maltose, but the broad range for this

disaccharide (1.37–4.96%) makes it of no special interest for the characterization of this class of honey.

Kojibiose was found at low levels in orange blossom and especially in sunflower honeys (1.0–2.45%), and at relatively high levels in honeydew honey (2.95–5.81%). Tukey's test leads to three non-overlaid groups: one for honeydew honey, another for orange and sunflower honeys and a third for the remaining honey varieties, with intermediate contents. From the results shown here, this disaccharide helps to differentiate orange and rosemary honeys, since the 95% confidence intervals for the mean do not overlap.

The distribution of maltulose is similar to that of kojibiose, although the concentrations were lower. Orange and sunflower honeys featured low levels of maltulose, whereas the highest levels were found in honeydew honey. The other honey types remained at an intermediate situation. Thus, three Tukey's groups are

Table 2. Distribution of the levels of various sugars (%), the fructose/glucose (F/G) and the glucose/water (G/W) ratios among seven types of Spanish unifloral honeys

Sugar	Rosemary (13)[a]	Orange blossom (16)	Lavender (15)	Sunflower (14)	Eucalyptus (14)	Heather (13)	Honeydew (16)
Fructose							
Mean	36.2	36.7	37.6	39.4	38.7	37.6	34.3
SD	1.6	2.0	0.9	0.7	0.6	1.2	1.1
Range	33.7–40.1	31.9–39.1	36.6–39.5	38.3–40.6	37.0–39.2	34.6–39.7	32.6–35.9
Glucose							
Mean	31.2	30.2	30.8	35.4	31.7	29.5	25.8
SD	2.3	2.3	2.2	1.4	1.4	1.4	1.5
Range	28.6–37.0	25.3–34.3	27.6–34.8	32.9–37.8	28.5–33.9	27.1–30.7	22.7–28.5
F/G ratio							
Mean	1.17	1.21	1.22	1.11	1.22	1.28	1.33
SD	0.10	0.05	0.08	0.04	0.05	0.07	0.06
Range	0.99–1.40	1.12–1.30	1.06–1.32	1.06–1.20	1.15–1.37	1.18–1.40	1.22–1.77
G/W ratio							
Mean	1.64	1.66	1.86	2.10	2.02	1.62	1.63
SD	0.14	0.16	0.15	0.18	0.14	0.08	0.10
Range	1.43–1.89	1.39–1.91	1.59–2.11	1.83–2.50	1.71–2.26	1.48–1.68	1.45–1.77
Sucrose							
Mean	1.6	4.45	0.82	0.073	0.30	0.062	0.21
SD	2.2	3.3	1.1	0.037	0.28	0.048	0.17
Range	0.045–5.7	1.05–12.0	0.044–3.72	0.032–0.15	0.07–0.94	0.025–0.21	0.02–0.75
'Maltose'[b]							
Mean	3.9	3.3	4.4	2.7	4.8	3.7	4.9
SD	0.7	1.0	0.6	0.3	0.7	0.6	0.8
Range	2.59–5.04	1.37–4.96	3.3–5.05	2.32–3.35	3.98–5.88	2.86–4.61	3.43–6.22
Maltulose							
Mean	1.63	0.92	1.75	0.75	1.62	1.93	3.35
SD	0.5	0.36	0.5	0.3	0.44	0.43	0.75
Range	0.96–2.55	0.25–1.30	0.98–2.64	0.46–1.49	0.83–2.20	1.11–2.59	2.51–5.28
Kojibiose							
Mean	2.4	1.73	2.6	1.55	2.7	2.4	3.8
SD	0.5	0.44	0.5	0.34	0.5	0.4	0.8
Range	1.80–3.20	ND–2.62	ND–3.00	1.00–2.45	ND–3.5	ND–3.13	2.95–5.81
Isomaltose							
Mean	0.97	0.45	0.97	0.29	0.73	0.93	1.8
SD	0.37	0.24	0.41	0.13	0.32	0.30	0.93
Range	0.56–2.00	0.13–1.16	0.13–1.4	0.17–0.68	0.31–1.42	0.43–1.39	0.45–4.5
Raffinose							
Mean	0.33	0.34	0.26	—	0.20	0.16	0.58
SD	0.13	0.19	0.32	—	0.12	0.27	0.35
Range	0.13–0.46	ND–0.60	ND–1.3	ND–0.1	ND–0.40	ND–0.96	0.0–1.26
Erlose							
Mean	0.54	0.41	0.39	ND	0.30	0.13	—
SD	0.53	0.23	0.37	—	0.12	0.22	—
Range	ND–2.1	ND–0.77	ND–1.21	ND	0.12–0.51	ND–0.56	ND–0.52
Melezitose							
Mean	—	—	—	ND	—	—	0.8
SD	—	—	—	—	—	—	0.69
Range	ND–0.20	ND–0.4	ND–0.1	ND	ND–0.24	ND–3.9	0.15–3.4

[a]Number of samples in parentheses.
[b]Includes the contribution of nigerose and turanose.
ND, not detected.

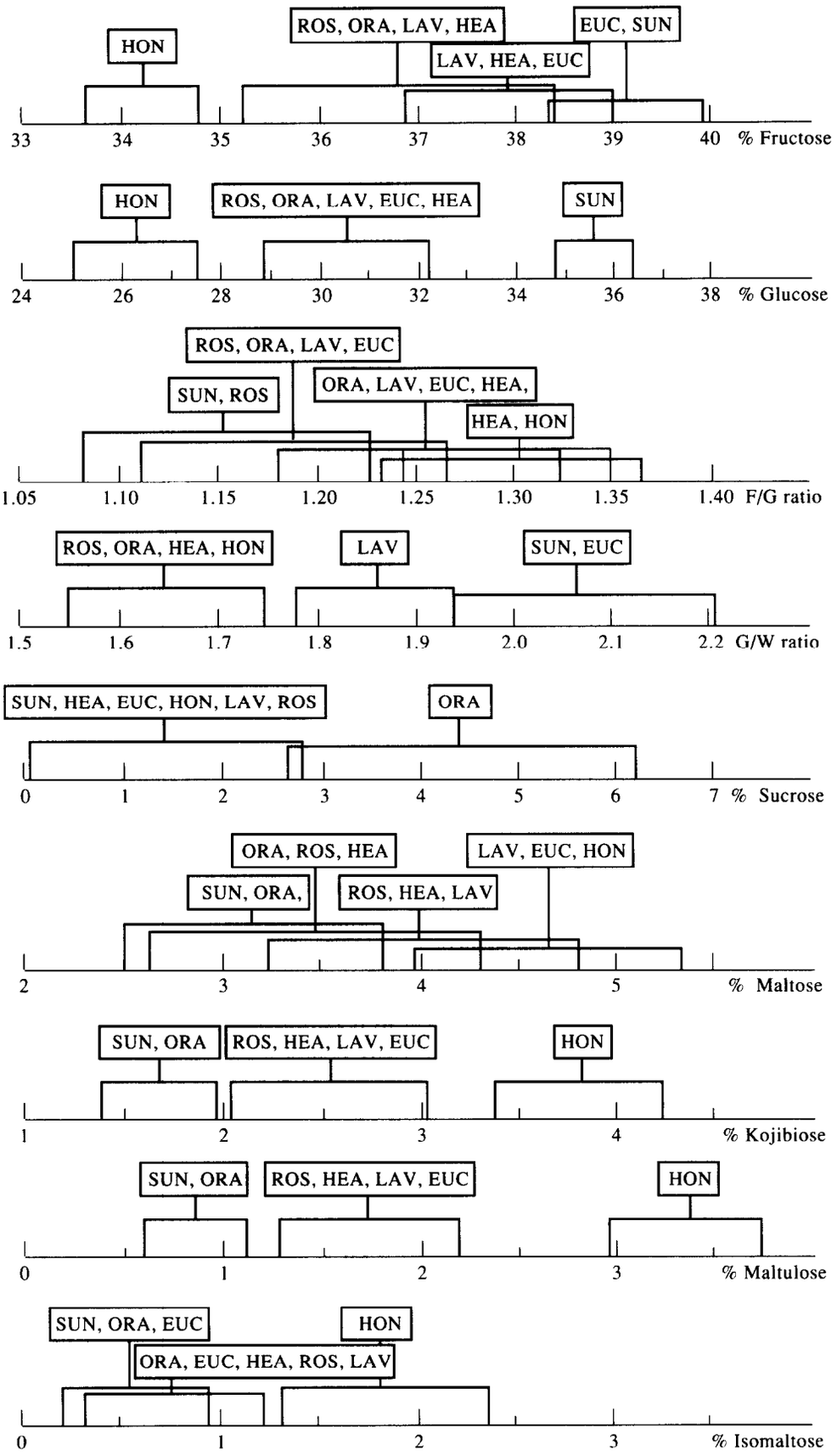

Fig. 2. Grouping of seven Spanish honey types based on Tukey's test ($P = 0.95$) for different sugar parameters. Each rectangle represents a honey group and its left and right sides are, respectively, the lowest and the highest 95% confidence limits for the mean of the honey types included in the group. The honey types are abbreviated as: ROS, rosemary; ORA, orange blossom; LAV, lavender; SUN, sunflower; EUC, eucalyptus; HEA, heather; HON, honeydew. F/G, fructose/glucose; G/W, glucose/water.

depicted in Fig. 1. The advantage of maltulose over kojibiose is the lack of possible interference from maltose, nigerose and turanose.

Honeydew honeys showed isomaltose concentrations significantly higher than floral honeys, and constitute a sole Tukey group. Sunflower honeys were low in this disaccharide too, with levels that were always below 0.7%.

With trisaccharides, limits of detection were about 0.03%. Undetectable levels have been considered as zero for calculation purposes. The levels found for raffinose were very variable except for sunflower honeys (only one sample contained raffinose at 0.1% level); it was detected in all rosemary honey samples (0.13–0.46%) but failed in many samples of the other varieties. Melezitose ranged from 0.15% to 3.4% in honeydew honey, but floral honeys did not present levels exceeding 0.4%. The exception was a heather honey sample (3.9%) where melezitose was present, perhaps as the result of contamination with honeydew. Erlose was detected in nearly all honey types (not in sunflower honeys), but it was not present in all samples within a honey type. This trisaccharide was found in all eucalyptus honey samples where it ranged from 0.12% to 0.51%. The highest value was found in rosemary honey (2.1%), but some samples contained undetectable levels of erlose, unlike reported values for French rosemary honeys (Institut Technique d'Apiculture, 1975). Sunflower honeys were very poor in trisaccharides; in fact, we did not detect erlose or melezitose in any sunflower honey sample.

Erlose is an intermediate trisaccharide in the metabolism of nectar sugars by honeybees; it is formed from sucrose by transglucosylation of the α-D-glucosyl group of a molecule of sucrose to the fourth position of the glucose moiety of another molecule of sucrose (White & Maher, 1953). Its level increases at first and then decreases by the action of honey α-glucosidase at the time new oligosaccharides are synthesized. The reported low concentration of sucrose in the nectar of Asteraceae (Baker & Baker, 1983) may be the reason for the undetectable levels of erlose in sunflower honey; it is consistent with the low levels of other oligosaccharides found in this type of honey. A relationship between low levels of sucrose in rape (Canola) flowers and the almost total absence of erlose in Canola honeys has been reported (Swallow & Low, 1990). Thus, botanical source of nectar seems to be of interest for the sugar profile of honeys, because other external factors affecting sunflower honey are the same (comb frame, beekeeping practices) or not especially different (ambient temperature and humidity of crop areas) from other types of summer Spanish honeys (namely lavender honeys, where 1.21% erlose level was reached).

Fructose, glucose, sucrose, 'maltose', and the G/W ratio were selected by the BMDP7M discriminant analysis program as the most valuable sugar parameters for establishing an accurate classification of the honey samples into their parent classes. Table 3 gives the classification matrix obtained by using these parameters as variables in the equations derived from the statistical program. All honeydew honey samples were correctly classified, which accounts for the interest in the sugars for characterization. Thus sugar analysis helps to differentiate between honeydew and heather honeys, which are rather similar in colour (Mateo *et al.*, 1992), pH and electrical conductivity (Bosch & Mateo, 1984). These sugar parameters also help to typify sunflower, heather and eucalyptus honeys (92.9%, 83.3% and 75.0% successful assignments, respectively), but their interest decreases for the remaining honey types because they only help to classify 53.8–69.2% of samples accurately. The average percentage of successful classifications (77.9%) indicates that the sugars selected by discriminant analysis can be considered, as a whole, valuable for the characterization of these unifloral honeys.

Other sugar parameters, although not selected by discriminant analysis, may be useful for the differentiation of two honey types when there is some doubt about its botanical origin; thus, as mentioned above, rosemary and orange honeys showed significantly different levels of maltulose and kojibiose although they are very similar in pH, electrical conductivity and colour (Bosch & Mateo, 1984; Mateo *et al.*, 1992).

Table 3. Classification matrix of Spanish unifloral honeys by sugar analysis using functions obtained by stepwise discriminant analysis[a]

Honey type[a]	Percent correct	Number of samples classified into type[b]						
		ROS	ORA	LAV	SUN	EUC	HEA	HON
ROS	69.2	9	2	1	0	0	1	0
ORA	66.7	0	10	3	1	0	1	0
LAV	53.8	0	0	7	1	2	3	0
SUN	92.9	0	0	0	13	0	1	0
EUC	75.0	0	0	3	0	9	0	0
HEA	83.3	2	0	0	0	0	10	0
HON	100.0	0	0	0	0	0	0	16
Total	77.9							

[a]The selected parameters were: fructose, glucose, sucrose, maltose and the glucose/water ratio.
[b]Types of honeys are abbreviated as: ROS, rosemary; ORA, orange blossom; LAV, lavender; SUN, sunflower; EUC, eucalyptus; HEA, heather; HON, honeydew.

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