Classification of Spanish Unifloral Honeys by Discriminant Analysis of Electrical Conductivity, Color, Water Content, Sugars, and pH

Rufino Mateo* and Francisco Bosch-Reig

Department of Analytical Chemistry, University of Valencia, Dr. Moliner 50, E-46100 Burjasot, Valencia, Spain

To ascertain the most discriminant variables for seven types of Spanish commercial unifloral honeys, stepwise discriminant analysis was performed. Fifteen parameters [pH; water content; electrical conductivity; *x*, *y*, and *L*, chromatic coordinates from the CIE-1931 (*xyL*) color space; fructose; glucose; sucrose; maltose; isomaltose; maltulose; kojibiose; and the fructose/glucose and glucose/water ratios] were considered. The studied honey types were rosemary, citrus, lavender, sunflower, eucalyptus, heather, and forest. The most discriminant variables, as selected by the multivariate program, were electrical conductivity, color (*x*, *y*, *L*), water content, fructose, and sucrose. All sunflower, eucalyptus, and honeydew honey samples and >90% of the samples from the remaining honey types were correctly classified by using the classification functions devised by the program. The overall proportion of accurately arranged samples was 95.7%. Results were validated by the "jackknifed" procedure and showed that electrical conductivity, color, water content, fructose, and sucrose are highly useful parameters to classify unifloral honeys, although microscopical analysis of honey sediment remains the fundamental tool.

Keywords: *Honey; discriminant analysis; electrical conductivity; color; water content; sugars; pH*

INTRODUCTION

Characterization of unifloral commercial honeys is a hard task initiated in Europe in response to consumer demands. Microscopical analysis, especially the identification and counting of pollen grains in honey sediment, is widely used to ascertain the botanical origin of honeys. Usually, honey is considered mainly from one plant (unifloral) if the pollen frequency of that plant is >45%. Pollen grains from anemophilous and nectarless plants are excluded in the calculation of the percentages. This rule is valid only if few honeydew elements (HDE) (which consist mainly of algae and fungal spores and hyphae) are present. Pollen grains from some taxa are "under- or over-represented" in relation to the nectar their flowers yield. For unifloral honeys with under-represented pollen, the minimum percentage of the taxon that gives the honey name is 10−20 or 20−30%. Sometimes pollen grains cannot be identified as far as the genus or species, and they may be associated in forms or types. Interpretation of pollen analysis data may be difficult in some cases, and the counting and identification of pollen grains depend greatly on the experience and performance of the operator (Maurizio, 1975; Louveaux et al., 1978).

Pourtallier and Taliercio (1970) suggested the use of physicochemical criteria such as sugar content, electrical conductivity, and pH analyses complemented by pollen analysis as the main criteria for characterization of unifloral honeys. In particular cases, they also suggested measurements of thixotropy and α-amylase activity. In recent years much work has been done to find chemical components of honeys originating in the nectar or produced by the bee through biochemical transformation of nectar compounds that could be used as markers for floral origin of honeys. Some of them are aroma compounds such as methyl anthranilate, which has been proposed as an indicator of citrus honey quality (Serra Bonvehí, 1988; Serra Bonvehí and Ventura Coll, 1995; White and Bryant, 1996), and other volatiles (Bouseta et al., 1996), amino acids (Davies, 1975; White and Rudgy, 1975; Bouseta et al., 1996) and their degradation products (Speer and Montag, 1987), aromatic acids and their esters (Speer and Montag, 1984; Steeg and Montag, 1988), aromatic and degraded carotenoid-like substances (Tan et al., 1988, 1989, 1990), and flavonoids such as hesperetine, a suggested marker for citrus honey (Ferreres et al., 1993, 1994a), and other flavonoids (Ferreres et al., 1994b).

The quality and composition standards of some unifloral French honeys have been reported (Institut Technique d'Apiculture, 1975). Kirkwood et al. (1960) used a linear function of pH and percentages of both ash and reducing sugars to differentiate between Australian honeys and honeydew honeys. Krauze and Zalewski (1991) used principal component analysis to evaluate some physicochemical parameters as tools for distinguishing honeys of different botanical origins. No single or small number of parameters have been described to characterize all of the large classes of unifloral honeys, and various determinations are needed, especially microscopical analysis of the sediment, which at present can be considered the reference method.

Most Spanish commercial unifloral honeys show problems in relation to pollen analysis related to under- and over-represented pollen grains. Rosemary, lavandin, and citrus honeys have relatively low under-represented pollen grains of the species/genus which give the honey name, and those samples with pollen

* Author to whom correspondence should be addressed (telephone 034-6-3864533; fax 034-6-3864436; e-mail Francisco.Bosch@UV.es).

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frequencies between 10 and 20% may be considered unifloral (Louveaux et al., 1978). Rather variable pollen content has been reported for rosemary, lavender, and citrus Spanish honeys (Serra Bonvehí et al., 1987; Mateo et al., 1992). The pollen from Spanish lavender (*Lavandula latifolia* Med.) honeys was considered as underrepresented, and a minimal percentage of 10−13% was suggested for unifloral honeys (Serra Bonvehí et al., 1987). Eucalyptus honeys usually display a high proportion of *Eucalyptus* spp. pollen grains, which may be considered over-represented (Ziegler et al., 1979; Pérez and Torreguitart, 1985; Serra Bonvehí, 1989; Mateo et al., 1992). Pollen analysis is not applicable to honeydew honeys, which instead contain many HDE and must have HDE/P (honeydew to pollen from nectar plants) ratios >3 to qualify as such (Louveaux et al., 1978). *Quercus* spp. honeydew honeys were studied by Serra Bonvehí et al. (1987) but their samples failed to fulfill this requirement.

Physicochemical parameters such as electrical conductivity and pH (Bosch and Mateo, 1984), color (Mateo et al., 1992), and sugar profiles (determined by gas chromatography) (Mateo and Bosch-Reig, 1997) were examined to test their suitability for characterizing Spanish unifloral honeys. Satisfactory results were obtained only for some honey types. Multivariate analysis was performed to assess differences in honey color due to its tristimular nature [three variables are used to determine honey color in CIE(*xyL*) or CIE(*Lab*) color spaces]. The results gave an overall proportion of 70.1−76.0% samples correctly classified into their parent classes (Mateo et al., 1992), which seems not very useful. Among sugars, the best discriminant parameters were fructose, glucose, sucrose, maltose and the glucose/water ratio. However, these sugars provided an overall percentage of correct classifications of only 71.6% (Mateo and Bosch-Reig, 1997).

The purpose of this work was the application of stepwise discriminant analysis to all of the parameters studied (pH, water content, electrical conductivity, color, sugars, and sugar ratios) in an effort to find the best combination to characterize seven Spanish unifloral honey types.

MATERIALS AND METHODS

Honey Samples. Honeys studied here account for ∼80% of the total honey production in Spain (Peris, 1984). Some of them are produced in relatively high amounts (sunflower, eucalyptus, rosemary); others, with lower yields, are highly appreciated for their pleasant flavor in Spain and/or abroad and are exported to other countries. The sources of honey samples and the methods to ascertain their botanical origin have been indicated previously (Mateo et al., 1992; Mateo and Bosch-Reig, 1997). The various samples of each honey type were harvested in different years between 1980 and 1987, and they were from different Spanish regions. Samples were screened by microscopical and sensory analysis assessment as soon as they arrived at the laboratory. When analysis had to be delayed for more than a month, they were stored at −20 °C; otherwise, they were stored at 4−6 °C in the dark. After pollen and sensory assessment, some samples within each type were selected as unifloral for further analysis. The honey samples used for all physicochemical determinations were as follows: 13 from rosemary (*Rosmarinus officinalis* L.); 16 from orange blossom (citrus) (*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, mainly *Erica* spp.); and 16 from honeydew (*Quercus* spp.). These last samples were assessed microscopically for pollen and HDE, as HDE/P ratios >3 are required for honeydew honeys (Louveaux et al., 1978); electrical conductivity values >800 μS/cm and pH values >4.3, besides acceptable sensory assessment, were also required for further consideration of these honeys (Vorwohl, 1964; Bosch and Mateo, 1984; Talpay, 1985).

Procedures. Microscopical analysis of the sediment was performed according to the methods of melissopalinology as given in Louveaux et al. (1978) as previously described (Mateo et al., 1992). Slides were prepared without acetolysis by centrifuging 10 g of honey dissolved in 20 mL of dilute sulfuric acid (5 g of H_2SO_4/L) for 10 min at 2500 rpm. The supernatant liquid was decanted, and the sediment was washed twice with 10 mL of distilled water and centrifuged. The sediment was put on a glass slide, sprouted over an area of 2 × 2 cm, dried at 40 °C, and mounted with stained glycerin−gelatin. Pollen grains were identified with the aid of our collection and microphotographs from specialized literature. After 300−400 grains were counted, they were classified in the following frequency classes: predominant pollen (>45% of the pollen grains counted); secondary pollen (16−45%); important minor pollen (3−15%), and minor pollen (<3%). Honeydew indicators were counted and the HDE/P ratios calculated.

Water content (moisture) was determined by refractometry according to AOAC methods (AOAC, 1980a) using a Bellingham and Stanley standard model Abbe-type refractometer.

Measurements of pH were performed potentiometrically at 20.0 °C in a 10% (w/v) solution of honey in freshly boiled distilled water using a Radiometer model 26 pH-meter (Radiometer, Copenhagen, Denmark) (Barbier and Pangaud, 1961; Pourtallier and Taliercio, 1970; Bosch and Mateo, 1984).

Electrical conductivity was measured at 20.0 °C in a 20% (w/v) solution of honey (dry matter basis) in deionized water with electrical conductivity <1 μS/cm (Vorwohl, 1964; Louveaux et al., 1973; Bosch and Mateo, 1984; Serra Bonvehí et al., 1987) using a Crison model 525 conductimeter (Crison Instruments, Barcelona, Spain).

Color of liquid honeys was determined by measurement of transmittances at 30 selected wavelengths, as previously described (Mateo et al., 1992), on a Shimadzu UV−vis 240 dual-beam spectrophotometer fitted to a chart recorder (Shimadzu Co., Tokyo, Japan). The *x*, *y*, and *L* chromatic coordinates from the CIE-1931(*xyL*) color system (CIE, 1931) were calculated from the tristimulus values and used for statistical treatment of the data.

Sugars were determined by gas chromatographic separation of the trimethylsilyl oximes and trimethylsilyl ethers (nonreducing sugars) in an OV-17 packed column on a Perkin-Elmer Sigma 3 gas chromatograph equipped with flame ionization detector (Perkin-Elmer Co., Norwalk, CT). Trimethylsilyl derivatives were obtained by reaction with hydroxylamine hydrochloride followed by derivatization with 1,1,1,3,3,3-hexamethyldisilazane and trifluoroacetic acid (Mateo and Bosch-Reig, 1997). The following sugars were determined: fructose, glucose, sucrose, "maltose", maltulose, kojibiose, and isomaltose. The term "maltose" includes true maltose, nigerose, and turanose. The fructose/glucose (F/G) and glucose/water (G/W) ratios were also calculated. Trisaccharides (raffinose, erlose, and melezitose) were not accurately determined or not detected, so they were not used in statistical calculations.

Statistical Analysis. The values of water content, pH, electrical conductivity, color (*x*, *y*, *L*), and sugar concentration of samples, as well as their F/G and G/W ratios, were statistically compared. Univariate analysis [one-way analysis of variance (ANOVA), Tukey's multiple-range test, Kolmogorov−Smirnov goodness of fit test to a normal distribution, Bartlett−Box test of homogeneity of variances] from the Statistical Package for Social Sciences package (SPSS, 1986) was used first to examine the reliability of the distributions obtained. Multivariate analysis (stepwise discriminant analysis, 7M) from the BMDP statistical package (Dixon et al., 1988) was then performed to determine the variables that best discriminate among honey types.

Not all analyzed samples could be used for discriminant analysis due to lack of specific values for some variables ("missing" values); hence, the real number of samples used for calculations was usually lower than the number of analyzed samples.

RESULTS AND DISCUSSION

The results of microscopical analysis of the sediment for the honeys used in this work are briefly summarized. Percentages are always referred to pollen from nectar plants. Rosemary honeys contained 20−77% pollen of *Rosmarinus officinalis*. Other taxa frequently identified were *Hypecoum* sp., Rosaceae (especially *Prunus dulcis*), Cruciferae type *Diplotaxis*, Cistaceae (*Cistus* spp., *Helianthemum* sp.), Leguminosae type *Ulex*, and *Thymus* sp. Other taxa were Boraginaceae (mainly *Echium* sp.), Compositae (mainly *Helianthus annuus*), *Erica* sp., *Olea europaea*, *Eucalyptus* sp., *Vitis vinifera*, Gramineae, *Rhamnus* sp., and other Leguminosae (*Onobrychis* sp., types *Genista*, *Trifolium*, or *Anthyllis*). Our results agree with data reported by other authors (Ricciardelli d'Albore and Vorwohl, 1979; Serra Bonvehí et al., 1987).

Citrus honeys contained 10−46% pollen of *Citrus* spp. although one sample reached 80% (which was explained by the proximity of hives to abandoned orange tree fields). Pollens of *O. europaea*, *Cistus* spp., *Quercus* sp., Crucifereae (especially type *Diplotaxis*), Compositae (mainly types *Taraxacum* and *Sonchus*), Leguminosae, Rosaceae, and Gramineae were frequent.

In lavender honeys the pollen of *L. latifolia* was secondary or predominant (two samples), ranging from 15 to 68%, which agrees with the findings of Serra Bonvehí et al. (1987). Lavender honeys contained also pollens of *H. annuus*, *Eucalyptus* sp., Compositae, Cistaceae, *Thymus* sp., Leguminosae (type *Onobrychis* and *Genista*) and *Hypecoum* sp. among others.

Sunflower honeys contained 45−82% pollen of *H. annuus*. Pollens of *Eucalyptus* sp., *Echium* sp., *Cistus* spp., Leguminosae, other Compositae, and Cruciferae were usually found as secondary or important minor.

Eucalyptus spp. pollen was always very predominant (82−98%) in eucalyptus honeys according to the reported over-representing presence of this pollen type (Ziegler et al., 1979; Pérez and Torreguitart, 1985; Serra Bonvehí, 1989). Other minor or important minor pollens were those from *Echium* sp., *Cistus* spp., Compositae, Ericaceae, and *Lavandula stoechas*.

Heather honeys contained 48−67% pollen from Ericaceae. Other taxa usually found were Cistaceae, *Eucalyptus* sp., *Echium* sp., *H. annuus*, Leguminosae, *Castanea sativa*, and *R. officinalis*.

The six honey classes mentioned above had practically no or few HDE; usually HDE/P ratios were lower than 0.15.

Honeydew honeys from *Quercus* spp. showed pollen spectra in which *Echium* sp. (probably *E. plantagineum*) was always present (it was predominant in 3 and secondary in seven samples). *C. sativa* (over-represented pollen) and Leguminosae type *Ulex* each were predominant in two samples, and no predominant pollen was found in the remaining samples. Other taxa usually found were *Cistus* spp., *Eucalyptus* sp., and Leguminosae types *Trifolium* and *Genista*. HDE/P ratios for these honey samples were variable (0.07−1.7) but lower than 3. Serra Bonvehí et al. (1987) report analogous problems and related them to mixing of honeydew honey from *Quercus* spp. with honey from previous blooming, due to beekeeping practices. Because of this problem they called their samples forest honeys. Piazza et al. (1986) found the same difficulties in Italian honeydew honeys. Thus, the intended classification of our samples as honeydew honeys becomes risky; classification as forest honeys, according to Serra Bonvehí et al. (1987), is advisable, even when oak forest honeys should be a less ambiguous name. The accepted samples agreed with pH, electrical conductivity, and sensory requirements. As reported by Maurizio (1975), the appearance of green algae in honey sediment seems to depend on climate factors and is often absent in honeydew honeys from dry areas such as *Quercus* species. This fact would account in part for the few HDE found as honeydew from oaks gathered by bees mainly in July and August in Extremadura and Salamanca (Western Spain), where the summer is very hot and dry.

Means, their standard errors (SEM), and ranges for 15 parameters determined in the subsets of unifloral honey samples with no "missing" values for any variable are listed in Table 1. For each parameter under consideration no significant differences (Student's t test, $P < 0.05$) were found between the means of these sample subsets and the means of the sets from which they were taken.

Data distributions within each type can be considered normal in general (Kolmogorov−Smirnov test, $P < 0.05$), but data variances (Bartlett−Box test, $P < 0.05$) for each parameter among all honey types are not always homogeneous. As deduced from application of ANOVA, differences among the seven honey types are significant ($P < 0.05$) for all of the parameters under consideration. Application of Tukey's test ($P < 0.05$) shows that honey classes can be grouped into several (two to five) groups depending on the parameter tested. Honey classes grouped together cannot be differentiated from each other but are statistically different from honey types belonging to other groups. Any honey type belonging to two groups cannot be considered statistically different from another one belonging to either of these groups. As indicated in Table 2, the numbers of groups are three for water content (two groups overlap), five for electrical conductivity, and four for pH (two groups overlap). Concerning CIE-1931 color coordinates, there are four groups for both x and L and three groups for y. In the case of sugars, the same grouping that was found for the original sample sets (Mateo and Bosch-Reig, 1997) was obtained: four groups for fructose (lavender and heather honeys are included in groups 2 and 3 and eucalyptus honeys in groups 3 and 4), two for sucrose (one for citrus honey and another for the remaining honeys), three for glucose, four for F/G ratio (two groups overlap), three for G/W ratio, four for maltose (one group overlaps with two others), three for kojibiose and maltulose, and three for isomaltose (two groups overlap). No variable in Table 2 can produce seven groups and separate adequately the honeys studied. However, electrical conductivity appears to be the most promising variable as it yields five groups, which agrees with the results of Krauze and Zalewski (1991).

The variables selected by stepwise discriminant analysis as the more discriminant were, in this order, electrical conductivity, y, x, L, fructose, water content, and sucrose. The stepwise process and the statistical parameters involved are shown in Table 3. At each step a one-way ANOVA was performed, and the variable with the highest F-to-enter value was selected.

Table 1. Distribution Data for Water Content, Electrical Conductivity, pH, Color (CIE-1931 *xyL*), and Sugars in the Subsets of Spanish Unifloral Honey Samples Used for Stepwise Discriminant Analysis

	honey type[a]						
parameter	rosemary (13)	citrus (15)	lavender (12)	sunflower (13)	eucalyptus (12)	heather (12)	forest (16)
water content (g/100 g)							
mean ± SEM[b]	19.1 ± 0.36	18.2 ± 0.26	16.6 ± 0.05	16.95 ± 0.28	15.7 ± 0.23	18.2 ± 0.15	15.8 ± 0.12
range	17.3−20.8	16.4−19.6	15.5−17.5	14.5−18.2	14.1−16.7	17.6−19.0	14.6−16.4
k[c] (μS/cm)							
mean ± SEM	172 ± 13	180 ± 9	269 ± 29	376 ± 16	465 ± 26	976 ± 29	986 ± 27
range	89−250	124−262	144−486	278−463	345−663	815−1092	822−1213
pH							
mean ± SEM	3.89 ± 0.05	3.96 ± 0.02	3.97 ± 0.05	3.88 ± 0.04	4.11 ± 0.05	4.45 ± 0.04	4.61 ± 0.04
range	3.68−4.20	3.83−4.06	3.68−4.31	3.61−4.20	3.82−4.35	4.30−4.70	4.40−4.97
x[d]							
mean ± SEM	0.370 ± 0.007	0.380 ± 0.006	0.469 ± 0.009	0.475 ± 0.003	0.458 ± 0.007	0.668 ± 0.015	0.592 ± 0.008
range	0.340−0.429	0.344−0.418	0.429−0.523	0.428−0.509	0.410−0.499	0.581−0.735	0.522−0.640
y[d]							
mean ± SEM	0.381 ± 0.006	0.393 ± 0.007	0.456 ± 0.003	0.478 ± 0.003	0.451 ± 0.005	0.330 ± 0.015	0.406 ± 0.004
range	0.352−0.435	0.356−0.436	0.434−0.469	0.450−0.488	0.419−0.481	0.265−0.417	0.359−0.452
L[d] (%)							
mean ± SEM	40.8 ± 6.4	66.0 ± 3.1	42.7 ± 3.2	49.8 ± 2.3	29.8 ± 1.9	1.8 ± 0.9	9.1 ± 1.3
range	34.12−80.84	44.94−84.12	23.62−60.56	31.01−61.91	20.34−38.23	0.002−9.89	2.84−24.28
fructose (g/100 g)							
mean ± SEM	36.2 ± 0.44	36.75 ± 0.54	37.4 ± 0.23	39.4 ± 0.19	38.7 ± 0.17	37.4 ± 0.32	34.3 ± 0.27
range	33.7−40.1	31.9−39.1	36.6−39.5	38.3−40.6	37.0−39.2	34.6−38.6	32.6−35.9
glucose (g/100 g)							
mean ± SEM	31.2 ± 0.44	30.4 ± 0.61	30.5 ± 0.73	35.2 ± 0.39	31.4 ± 0.40	29.5 ± 0.43	25.8 ± 0.38
range	28.6−37.0	25.3−34.0	27.6−34.8	32.9−37.8	28.5−33.1	27.1−30.7	22.7−28.8
fructose/glucose							
mean ± SEM	1.17 ± 0.032	1.20 ± 0.023	1.23 ± 0.023	1.12 ± 0.011	1.23 ± 0.016	1.27 ± 0.020	1.33 ± 0.015
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
glucose/water							
mean ± SEM	1.63 ± 0.032	1.68 ± 0.042	1.83 ± 0.045	2.08 ± 0.050	2.01 ± 0.041	1.62 ± 0.023	1.63 ± 0.25
range	1.43−1.89	1.39−1.91	1.59−2.19	1.83−2.50	1.71−2.26	1.48−1.68	1.45−1.77
sucrose (g/100 g)							
mean ± SEM	1.60 ± 0.50	4.24 ± 0.85	0.88 ± 0.33	0.073 ± 0.010	0.29 ± 0.082	0.062 ± 0.015	0.21 ± 0.04
range	0.045−5.70	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[e] (g/100 g)							
mean ± SEM	3.90 ± 0.17	3.45 ± 0.24	4.40 ± 0.17	2.74 ± 0.08	4.81 ± 0.21	3.60 ± 0.15	4.90 ± 0.20
range	2.59−5.04	1.37−4.96	3.30−5.05	2.32−3.35	3.98−5.88	2.86−4.61	3.43−6.22
maltulose (g/100 g)							
mean ± SEM	1.63 ± 0.11	0.90 ± 0.10	1.63 ± 0.16	0.77 ± 0.09	1.65 ± 0.12	1.91 ± 0.13	3.35 ± 0.19
range	0.96−2.55	0.25−1.30	0.98−2.50	0.46−1.49	0.90−2.20	1.11−2.59	2.51−5.28
kojibiose (g/100 g)							
mean ± SEM	2.40 ± 0.11	1.73 ± 0.21	2.69 ± 0.24	1.58 ± 0.096	2.7 ± 0.15	2.4 ± 0.11	3.8 ± 0.20
range	1.80−3.20	1.14−2.62	1.79−3.00	1.00−2.45	1.65−3.50	2.01−3.13	2.95−5.81
isomaltose (g/100 g)							
mean ± SEM	0.97 ± 0.08	0.47 ± 0.065	1.09 ± 0.11	0.31 ± 0.04	0.79 ± 0.086	1.00 ± 0.07	1.8 ± 0.23
range	0.56−2.00	0.13−1.16	0.13−1.40	0.17−0.68	0.31−1.42	0.46−1.39	0.45−4.5

[a] The number of samples of each honey type (n) is given in parentheses. [b] Standard error of the mean (SD/$n^{1/2}$). [c] Electrical conductivity. [d] Chromatic coordinates in the CIE-1931 (xyL) color space. [e] This term accounts for maltose plus nigerose and turanose.

The discriminant program calculates the canonical correlations between the variables entered and the dummy variables representing the groups, and the coefficients for the canonical variables. A canonical variable (sometimes called the Fisher linear discriminant function) is a linear function of the variables selected by the program which discriminates among the groups. It maximizes the ratio of the between-group sum of squares to the within-group sum of squares. If the number of groups considered is p, there are $p - 1$ canonical variables that are orthogonal. Canonical variables are adjusted so that the (pooled) within-group variances are 1 and the overall mean is 0. The first canonical variable (that which accounts for the highest between-group variability) is that which best discriminates among the groups. The second is the next best linear function orthogonal to the first canonical variable and accounts as best as possible for differences among groups not shown by this one, etc. (Afifi and Azen, 1979; Dixon et al., 1988; Morrison, 1990; Manly, 1994). Table 4 lists the cumulative proportion of total dispersion, the canonical correlations, and the standardized coefficients for the six canonical variables. The higher is the absolute value of a standardized coefficient, the more significant is the related selected variable in the canonical variable. Electrical conductivity appears to be the parameter that contributes most to the first canonical variable (standardized coefficient = −0.754), which accounts for most of the discrimination between groups (66.27%). The second canonical variable is very related with color and fructose content, as deduced from the high absolute values of the standardized coefficients for x, y, L, and fructose (−1.206, −1.067, −0.796, and −0.687, respectively). The third canonical variable is dependent mainly on water content and x, and, secondarily, on fructose content. The three first canonical variables can explain up to 94.6% of the total dispersion, and the remaining are not very significant.

The general shape of the distribution of all sample scores on a scatter diagram whose axes are the first two canonical variables is shown in Figure 1.

Table 2. Grouping of Spanish Unifloral Honeys in Relation to Different Variables by Tukey's Test ($P < 0.05$)

variable	honey group[a]				
	group 1	group 2	group 3	group 4	group 5
water content	EUC, FOR	FOR, LAV, SUN	HEA, CIT, ROS		
k[b]	ROS, CIT	LAV	SUN	EUC	HEA, FOR
pH	ROS, SUN, LAV, CIT	CIT, EUC	HEA	FOR	
x[c]	ROS, CIT	EUC, LAV, SUN	FOR	HEA	
y[c]	HEA	ROS, CIT, FOR	EUC, LAV, SUN		
L[c]	HEA, FOR	EUC	LAV, SUN	ROS, CIT	
fructose	FOR	ROS, CIT, LAV, HEA	LAV, HEA, EUC	EUC, SUN	
glucose	FOR	HEA, CIT, LAV, ROS, EUC	SUN		
sucrose	HEA, SUN, FOR, EUC, LAV, ROS	CIT			
maltose	SUN, CIT	CIT, HEA, ROS	HEA, ROS, LAV	LAV, EUC, FOR	
kojibiose	SUN, CIT	ROS, HEA, LAV, SUN, EUC	FOR		
maltulose	SUN, CIT	ROS, HEA, LAV, SUN, EUC	FOR		
isomaltose	SUN, EUC, CIT	CIT, ROS, HEA, LAV	FOR		
F/G[d]	SUN, ROS	ROS, EUC, CIT, LAV	EUC, CIT, LAV, HEA	FOR	
G/W[e]	HEA, FOR, ROS, CIT	LAV	SUN, EUC		

[a] Honey types are abridged as ROS (rosemary), CIT (citrus), LAV (lavender), SUN (sunflower), EUC (eucalyptus), HEA (heather), and FOR (forest). [b] Electrical conductivity. [c] Chromatic coordinates in the CIE-1931 (xyL) color space. [d] Fructose/glucose ratio. [e] Glucose/water ratio.

Table 3. Results of Stepwise Discriminant Analysis of 15 Variables in Spanish Unifloral Honeys

step	selected variable	F value to enter	U-statistic (Wilks' λ)	approximate F statistic	degrees of freedom	
1	electrical conductivity	255.07	0.053203	255.072	6	86.00
2	y[a]	36.91	0.014757	102.449	12	170.00
3	x[a]	25.45	0.005236	71.472	18	238.07
4	L[a]	16.47	0.002390	56.241	24	290.76
5	fructose	14.95	0.001141	48.843	30	330.00
6	water content	12.08	0.000602	43.919	36	358.46
7	sucrose	9.52	0.000351	40.124	42	378.87

[a] Chromatic coordinates in the CIE-1931 (xyL) color space.

Classification functions are linear combinations of the variables selected by the program and have the form

$$Z_i = a_{i1}V_1 + ... + a_{ij}V_j + ...+ a_{ip}V_p + c_i \qquad (1)$$

with $i = 1, ..., k$ and $j = 1, ..., p$ ($p = k = 7$ in this case)

where Z_i is the ith linear discriminant score; a_{ij} are coefficients for the honey type i and the variable j. A constant for each honey type is denoted c_i. V_j represents the value of the variable j. The coefficients and constants for these functions appear in Table 5.

Classification functions can be used to predict the probability for the inclusion of a new sample m whose group membership is unknown into one of the seven honey types studied. To this aim, seven Z_{mi} scores were calculated with the coefficients from Table 5 and the values of the appropriate variables, experimentally obtained. The sample was assigned to the honey class for which the classification function has the largest Z_{mi} score. The probability $\Pr(W_i/m)$ of including the sample m into each of the seven unifloral honey groups (W_j) is given by (Afifi and Azen, 1979)

$$\Pr(W_i/\mathrm{m}) = \mathrm{e}^{Z_{mi}}/\sum_{j=1}^{k} \mathrm{e}^{Z_{mj}} \qquad (2)$$

The normal classification matrix is shown in Table 6. It gives the number of samples classified into each honey type and the percentages of successful classification. As cited earlier some samples were not computed due to "missing" values; thus, the number of samples in Table 6 is lower than the number of samples analyzed.

All sunflower, eucalyptus, and forest honey samples were correctly classified into their a priori established honey types. Rosemary, citrus, lavender, and heather honeys accounted for 92.3, 93.3, 91.7, and 91.7% correct classifications, respectively. The overall percentage of correct classification was 95.7%. Predictability of the procedure may be tested by cross-validation methods. One of these approaches is called the "leave-one-out" or "jackknife" method (Lachenbruch and Mickey, 1968). The nearly unbiased jackknifed matrix (which results when classification of any particular sample is achieved by considering all of the remaining samples but excluding the contribution of the sample being classified) differed only slightly from the normal matrix and gave an overall value of 93.5% correct classifications (100% for sunflower, eucalyptus, and forest honeys and 92.3, 86.7, 83.3, and 91.7% for rosemary, citrus, lavender, and heather honeys, respectively). Interestingly, differences in pollen spectra and HDE did not produce inconsistent results in forest honeys. This high degree of successful classification accounts for the ruggedness of the variables selected and validates them. Regardless of the jackknife method, the classification functions in Table 5 were applied to six samples of unifloral honeys (one citrus, two lavender, two eucalyptus, and one heather honey), which were not computed by discriminant analysis due to missing values for other variables. Five of them were correctly classified, but a lavender sample (18% pollen of *L. latifolia* and 3% pollen of *H. annuus*) failed and was classified as sunflower honey.

The following samples were misclassified in the normal matrix:

One rosemary honey sample was classified as lavender honey. It contained 47% *R. officinalis* pollen and about 3% *L. latifolia* pollen. Its atypical dark-amber color ($x = 0.429$, $y = 0.435$, $L = 47.79\%$) may be the cause for its unsuccessful classification.

One citrus honey sample was classified as rosemary honey, However, the proportion of pollen from *R. officinalis* was ~3%, whereas that from *Citrus* spp. was 41%. Its lightness ($L = 44.94\%$) and sucrose content (1.04 g/100 g) were the lowest in the subset of citrus honeys and may explain this misclassification. The posterior probabilities (eq 2) for classifying it as rosemary and orange honeys were 0.646 and 0.353, respectively.

Table 4. Cumulative Proportion of Total Dispersion, Canonical Correlations, and Standardized Coefficients for Canonical Variables Obtained by Discriminant Analysis of Water Content, Electrical Conductivity, pH, Color (CIE-1931, *xyL*), and Sugars in Spanish Unifloral Honeys

	canonical variable					
	1	2	3	4	5	6
cumulative proportion of total dispersion	0.6627	0.8530	0.9456	0.9693	0.9887	1.0000
canonical correlations	0.9782	0.9296	0.8694	0.6653	0.6273	0.5231
selected variables						
water content	0.294	0.035	−0.732	0.095	0.012	0.518
electrical conductivity	−0.754	0.073	0.047	−0.123	−0.824	0.428
fructose	0.467	−0.687	−0.576	−0.650	−0.655	0.050
sucrose	0.381	−0.293	−0.446	−0.115	−0.717	−0.692
x^a	−0.079	−1.206	−0.635	0.589	0.577	−0.437
y^a	0.257	−1.067	0.180	0.293	−0.087	0.264
L^a	0.357	−0.796	−0.292	0.988	−0.319	0.114

[a] Chromatic coordinates in the CIE-1931 (*xyL*) color space.

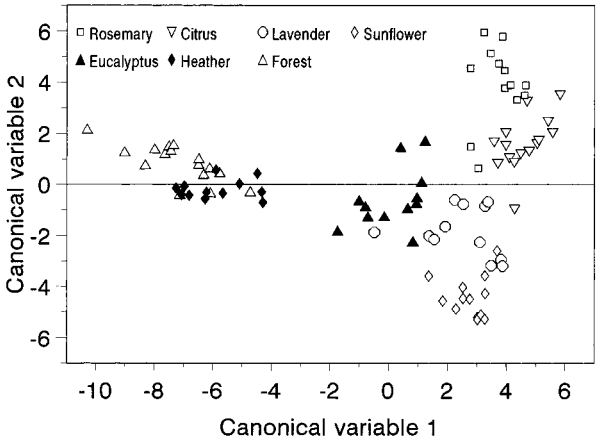

Figure 1. Discriminant analysis of some Spanish unifloral honeys as shown by a scatter diagram representing the projections of the points of each unifloral honey sample on the plane formed by the two principal canonical variables.

One sample of lavender honey was classified as sunflower honey. It contained 19% *L. latifolia* pollen, but the proportion of *H. annuus* pollen was similar (17%). This sample may be considered a mixture of both lavender and sunflower honeys, which occurs due to contemporary flowering of both species in the same crop areas; however, its high fructose level (39.5 g/100 g) and *L* value (54.68%) put it into the sunflower class, yet the sucrose content (0.56 g/100 g) is >3 times the maximum level reached by sunflower honeys (0.15 g/100 g) (Table 1). The decision is problematic as premium grade lavender honeys are more appreciated and expensive than sunflower honeys.

Finally, one sample of heather honey was assigned to the honeydew honey class. This sample had the lowest values for *x* and fructose and the highest values for *y*, *L*, and sucrose, among Ericaceae honeys, as well as an unexpectedly high proportion of trisaccharides, especially melezitose (3.9 g/100 g), which points to contamination with honeydew. This mix cannot be suspected from pollen analysis (52% Ericaceae pollen, HDE/P = 0.11) or from pH (4.54) and electrical conductivity (860 μS/cm), which are rather similar in heather and honeydew honeys (Table 1).

To confirm the ruggedness of the selected variables, stepwise discriminant analysis was also performed after the misclassified heather honey sample and some rosemary, citrus, lavender, and sunflower honeys (whose floral origin might be questionable on the basis of low pollen count) were discarded. In this case, the same variables were also selected and the percentages of correct assignments were 100% for sunflower, eucalyptus, heather, and honeydew honey and 80−92% for the three remaining honey types. No inconsistency over the validity of the variables selected as more discriminant was found by elimination of these samples. On the basis of these results, our data showed that the determinations of electrical conductivity, color (*x*, *y*, and *L* chromatic coefficients), water content, fructose and sucrose were very useful tools for the characterization of the honey types studied. Moreover, these determinations were achieved easily. Other available methods can be used to evaluate fructose, and sucrose in honey (Bergmeyer and Bernt, 1974; Palmer and Brandes, 1974; Zürcher et al., 1975; Thean and Funderburk, 1977; Deifel, 1985; Bogdanov and Baumann, 1988; AOAC, 1990). The partially successful classification of these honeys into their parental types, described in other papers (Mateo et al., 1992; Mateo and Bosch-Reig, 1997), has been notably improved in this study.

The experimental data for the variables selected by discriminant analysis have been contrasted with those available in the literature for Spanish unifloral honeys. In general, our results for water, fructose, and sucrose contents and electrical conductivity agree well (Welch test for comparison of means, $P < 0.01$) with data for rosemary, citrus, lavender, eucalyptus, and honeydew honeys (Serra Bonvehí et al., 1987; Serra Bonvehí, 1989; Pérez-Arquillué et al., 1994). However, there was a significant difference between electrical conductivity means in lavender honeys. In this case, our results agreed better with those reported for French lavender honeys (mean value = 250 μS/cm) (Institut Technique d'Apiculture, 1975). Data for water, fructose, and sucrose contents from fresh citrus honeys (Serra Bonvehí and Ventura Coll, 1995) did not agree with our results, which were mainly from mature citrus honeys. No disagreements between our mean values and those from Serra Bonvehí (1989) are found for *x* and *y* chromatic coordinates in eucalyptus honeys.

Some degree of doubt about the correctness of arrangements based on linear classification functions (Table 5) may remain despite using the seven more significant variables, especially in the case of rosemary, citrus, lavender, or heather honeys. It seems convenient to use other available variables to complement and confirm the provisional assignment of a sample to a group. These variables may be pH, other sugars (glucose, maltose, maltulose, kojibiose, glucose/water ratio, trisaccharides), proline, acidity (Mateo and Bosch-

Table 5. Coefficients for Classification Functions of Spanish Unifloral Honeys[a]

	parameters							
honey type	water content	k^b	fructose	sucrose	x^c	y^c	L^c	constant
rosemary	59.01	−0.0538	64.14	32.790	2327.0	2444.0	7.136	−2853.5
citrus	58.75	−0.0547	66.37	34.910	2430.0	2536.0	7.494	−3034.0
lavender	56.38	−0.0638	65.67	33.530	2564.0	2648.0	7.534	−3067.0
sunflower	58.57	−0.0412	68.58	34.600	2639.0	2766.0	7.941	−3332.0
eucalyptus	54.89	−0.0188	65.79	33.213	2402.5	2574.5	7.153	−2942.0
heather	57.70	0.0273	64.81	32.855	2522.0	2478.0	7.171	−3015.0
forest	52.82	0.0505	60.84	30.875	2390.0	2479.0	7.033	−2729.0

[a] For correct use of the coefficients, water, fructose, and sucrose contents have to be expressed in g/100 g of honey, and electrical conductivity in μS/cm. [b] Electrical conductivity. [c] Chromatic coordinates in the CIE-1931 (*xyL*) color space.

Table 6. Classification Matrix of Spanish Unifloral Honeys on the Basis of Electrical Conductivity, Color, and Water, Fructose, and Sucrose Contents

honey	%	number of samples classified into honey type[a]						
type[a]	correct	ROS	CIT	LAV	SUN	EUC	HEA	FOR
ROS	92.3	12	0	1	0	0	0	0
CIT	93.3	1	14	0	0	0	0	0
LAV	91.7	0	0	11	1	0	0	0
SUN	100	0	0	0	13	0	0	0
EUC	100	0	0	0	0	12	0	0
HEA	91.7	0	0	0	0	0	11	1
FOR	100	0	0	0	0	0	0	16
total	95.7	13	14	12	14	12	11	17

[a] Honey types are abridged as ROS (rosemary), CIT (citrus), LAV (lavender), SUN (sunflower), EUC (eucalyptus), HEA (heather), and FOR (forest).

Reig, 1997; Krauze and Zalewski, 1991), methyl anthranilate, or hesperetin, in the case of citrus honeys (Serra Bonvehí and Ventura Coll, 1995; White and Bryant, 1996; Ferreres et al., 1993; 1994a) or some volatiles for eucalyptus and lavender honeys (Bouseta et al., 1996). Research on unequivocal markers that can be readily and easily determined should continue.

Microscopical analysis must be kept as a tool of primary interest. However, its conclusions cannot be regarded as entirely decisive in determining the botanical origin of the unifloral Spanish honeys studied. Microscopical analysis needs to be complemented by consistent chemical or physicochemical determinations, especially for honey samples with pollen percentages near or between the limits to be considered as unifloral.

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