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Characterisation of Moroccan unifloral honeys by their physicochemical characteristics

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Abstract

Physicochemical parameters of 98 samples of Moroccan honeys were analysed; nine parameters were measured, including water content, pH, acidity (free, lactonic, total and lactonic acidity/free acidity ratio), hydroxymethylfurfural, diastase activity and proline. In addition, characterisation of the five unifloral honeys (*Eucalyptus* sp., *Citrus* sp., *Lythrum* sp., Apiaceae and honeydew) by principal component analysis (PCA) and stepwise discriminant analysis (SDA) was carried out. PCA showed that the cumulative variance was approximately 62%, and about 82% of the samples were correctly classified by using the stepwise discriminant analysis, with the best results being obtained for the eucalyptus and honeydew honeys (100% correct).

Keywords: Moroccan unifloral honey; Physicochemical parameters; PCA; SDA

1. Introduction

Usually, a honey is considered mainly from one plant (unifloral) if the pollen frequency of that plant is >45%. Pollen grains from 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%; for unifloral honeys with over-represented pollen, the minimum percentage of the taxon that gives the honey name is 70–90%.

Some unifloral honeys have specific chemical or physical properties, which may be used to confirm the results of microscopical analysis. For instance, the thixotropy and protein content of (heather) honey can be measured by simple methods; acacia and tupelo honeys contain much fructose; honeydew honeys have a high electrical conductivity; some honeydew honeys contain much melezitose; methyl anthranilate is considered as an indicator of citrus honey (Serra Bonvehí & Ventura Coll, 1995).

Some authors, such as Kirkwood, Mitchell, and Smith (1960), characterised honeydew honeys by a discriminant function based on the pH value and the percentages of ash and reducing sugars. Mateo and Bosch-Reig (1997) used only the sugar profiles for classifying Spanish unifloral honeys by discriminant analysis. Other authors, have characterised Spanish unifloral honeys by a discriminant analysis using the following parameters: pH, water content, sugars, colour and electrical conductivity (Mateo & Bosch-Reig, 1998).

In spite of the well-rooted honey tradition in Morocco, the few references that can be found about honey studies [Damblon, Fraval, Mtargi, Tazi, & Zbair, 1991; Lobreau-Callen & Damblon, 1994; Terrab, Valdés, & Díez, 2001, in press] refer to pollen analysis and, for physicochemical studies, only one work about the sugar profile is known (Terrab, Vega-Pérez, Díez, & Heredia, 2001). Thus, the goal of this work can be summarised under two headings: (1) the study of the water content, pH, acidity, HMF, diastase activity and proline of Moroccan honeys; (2) to apply different statistical methods in an effort to find the best combination to characterise five Moroccan unifloral honey types.

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2. Materials and methods

2.1. Honey samples

The present study was carried out using 98 honeys from northwest Morocco; the samples were collected directly from professional beekeepers and the extracted honeys were attained by centrifugation.

2.2. Pollen analysis

For the quantitative analysis, the method described by Maurizio (1979) was followed, where all the elements of botanical origin were counted from a sub-sample of 10 g of honey. For the qualitative analysis, acetolysed slides were made (Erdtman, 1960). According to the results obtained by Montero and Tormo (1990) and Saá-Otero, Díaz, and González (1993), at least 400 pollen grains were counted and identified. For the pollen identification, the general key to pollen types from Díez (1987) was used, giving 59 multifloral and 39 unifloral samples. The unifloral honeys were as follows: 12 from *Eucalyptus* sp., 10 from *Citrus* sp., 7 from *Lythrum* sp., 7 from Apiaceae and 3 from honeydew.

2.3. Physicochemical parameters

Water content (moisture) was determined with an Erma refractometer reading at 20 °C, using the Wedmore table (AOAC, 1990).

pH was measured by a pH-meter (Orion 420 A) in a solution containing 10 g honey in 75 mL of CO_2 free distilled water (AOAC, 1990).

The free, lactonic and total acidity were determined as follows, by the titrimetric method: the addition of 0.05 M NaOH is stopped at pH 8.50 (free acidity), immediately a volume of 10 ml 0.05 M NaOH is added and, without delay, back-titrated with 0.05 M HCl to pH 8.30 (lactonic acidity). Total acidity results are obtained by adding free plus lactone acidities (AOAC, 1990).

Hydroxymethylfurfural (HMF) was determined after clarifying samples with Carrez reagents (I and II) and the addition of sodium bisulfate (AOAC, 1990); absorbance was determined at 284 and 336 nm in a 1 cm quartz cuvette in a spectrophotometer (Milton Roy UV–vis Spectronic 3000 Array).

Diastase activity was measured using a buffered solution of soluble starch and honey, which was incubated in a specially designed glass tube (the end is shaped as an inverted "V") in a thermostatic bath until the endpoint was determined photometrically (AOAC, 1990).

Proline was determined according to the AOAC method, based on the reaction of the proline with ninhydrin in an acidic medium and measurement of the resulting product by the absorbance at 517 nm (AOAC, 1990).

2.4. Statistical analysis

Multivariant statistical treatments were carried out using Statistica® (Statsoft, 1999).

3. Results and discussion

3.1. Pollen analysis

The results of microscopical analysis of the sediment for the honeys used in this work are briefly summarised. Percentages are always referred to pollen from nectar plants. *Eucalyptus* sp. pollen was always very predominant (70–99%) in eucalyptus honeys according to the reported over-representing presence of this pollen type (Serra Bonvehí, 1989; Seijo, Aira, & Jato, 1998). Orange honeys contained 10–53% pollen of *Citrus* sp. The loosestrife honeys contained 46–86% pollen of *Lythrum* sp.; and Apiaceae honeys contained: 59–71% pollen of *Ammi visnaga*, 54–56% pollen of *Eryngium campestre*, and 45–60% pollen of *Ridolfia segetum*. The honeydew honeys present a HDE/NPGN (honeydew elements/number of pollen grains from nectariferous species) ratio greater than 1, with electrical conductivity values over 1400 S/cm.

3.2. Physicochemical parameters

Table 1 shows the means, standard deviations and ranges of the different physicochemical parameters. Water content, a parameter related to the maturity degree (White, 1975), shows values between 14 and 21.3%; two samples with a water content over 20% were found, the maximum allowed by the Spanish regulations (Anonymous, 1983), and by the European Community (El Consejo de la Unión Europea, 2002).

pH values, of great importance during honey extraction and storage, due to influence on texture, stability and endurance, range between 2.25 and 4.71. Owing to the presence of organic acids, in equilibrium with their corresponding lactones, or internal esters, and some inorganic ions such as phosphate and sulphate, the values for the free acidity ranged from 10.31 to 102 meq/kg; the lactonic acidity (considered as the acidity reserve when the honey becomes alkaline) ranges between 0.01 and 21.4 meq/kg, while the total acidity ranges between 12.6 and 115 meq/kg. Five of the samples surpassed the limit allowed by the European Community regulations (El Consejo de la Unión Europea, 2002), i.e. 50 meq/kg.

The hydroxymethylfurfural content, an indicator of honey freshness (Schade, Marsh, & Eckert, 1958), show values between 3.2 and 52.6 mg/kg, although four samples were found to surpass the limit permitted by the

Table 1
Distribution data for physicochemical parameters in Moroccan honey samples

Parameter	Honey type[a]					
	Eucalyptus (12)	*Citrus* (10)	*Lythrum* (7)	Apiaceae (7)	Honeydew (3)	Multifloral (59)
Water content (%)						
Mean±SD[b]	17.3±1.5	16.8±1.9	16.8±0.9	17.6±1.77	20.3±3.7	17.59±1.88
Range	15.5–19.37	14.5–21.3	15.6–18.3	15.0–20.0	16.2–23.6	13.10–24.10
pH						
Mean±SD	3.65±0.5	3.55±0.35	3.62±0.22	3.99±0.36	4.28±0.39	3.72±0.38
Range	2.25–4.17	3.01–4.09	3.25–3.92	3.58–4.58	3.92–4.71	2.61–4.55
Free acidity (meq/Kg)						
Mean±SD	19.5±5.31	20.8±10.9	28.4±5.36	30.0±10.7	88.6±23.4	29.8±10.7
Range	10.3–27.0	10.6–39.7	22.60–35.8	14.6–42.7	61.5–102	12.0–61.3
Lactonic acidity (meq/Kg)						
Mean±SD	9.26±4.86	9.31±5.03	7.58±5.67	12.1±6.84	8.08±4.87	12.1±4.51
Range	0.5–15.0	1.15–15.1	1.44–17.5	2.30–18.5	3.25–13.0	0.01–21.4
Total acidity (meq/Kg)						
Mean±SD	28.7±8.83	30.1±11.7	36.0±8.79	42.1±12.9	96.7±24.0	41.9±12.4
Range	15.4–38.8	12.6–44.7	27.6–53.3	28.1–59.9	69.5–11.5	14.3–70.4
Lactonic acidity/Free acidity						
Mean±SD	0.47±0.24	0.56±0.39	0.26±0.18	0.47±0.39	0.09±0.05	0.44±0.21
Range	0.01–072	0.04–1.17	0.04–0.53	0.08–1.26	0.03–0.13	0.01–1.23
HMF (mg/Kg)						
Mean±SD	16.1±11.2	17.7±12.5	7.92±2.37	9.98±6.09	31.7±19.73	17.5±9.10
Range	3.20–52.60	5.01–43.3	5.5–12.4	3.20–20.0	13.40–52.6	3.80–48.4
Diastase activity (°Gothe)						
Mean±SD	40.5±39.0	40.2±89.2	34.7±24.9	35.4±34.7	11.2±2.60	27.6±32.6
Range	9.50–158.0	1.63–290	8.45–85	8.40–108	8.60–13.8	0.18–236
Proline (mg/100 g)						
Mean±SD	56.09±14.1	25.0±9.42	38.5±23.1	63.9±41.5	227±80.4	74.1±37.9
Range	31.6–77.0	15.8–43.6	17.0–81.6	17.2–145	142–301	1.58–203

[a] The number of samples of each honey type (*n*) is given in parentheses.
[b] Standard deviation.

Spanish quality standards (Anonymous, 1983) and the European Community regulations (El Consejo de la Unión Europea, 2002; 40 mg/kg); this does not represent a sanitary problem, in fact, some authors (White, 1994) have proposed the increase of this permitted limit up to 80 mg/kg.

Diastase activity shows very different values, ranging between 0.18 and 236°Gothe, and only four samples show values below 8°Gothe, the limit allowed by the European Community regulations (El Consejo de la Unión Europea, 2002).

Different aminoacids can be found in honey, for example, lysine, histidine, arginine, aspartic acid, threonine and serine, proline being the most important from a quantitative point of view, since it is present with a mean percentage of 50% in relation to the rest (Komanine, 1960). In this study, the values for proline range between 1.58 and 300 mg/100 g.

3.2.1. *Eucalyptus (*Eucalyptus *sp.)*

The eucalyptus honeys mainly come from *Eucalyptus camaldulensis* Dehnh, considered the most important honey in Morocco and North Africa from a quantitative point of view; it is a medium amber, dense honey, with a pleasant mild woody flavour, that granulates fairly slowly with large brown grains. This type of honey shows similar values for water content ($\bar{x}=17.3\%$), acidity (free acidity $\bar{x}=19.5$ meq/kg) and HMF ($\bar{x}=16.1$ mg/kg) as those reported by Serra Bonvehí (1989) in Spanish eucalyptus honeys, and a relatively high value for the diastase activity ($\bar{x}=16.1$°Gothe). Proline showed a medium value of 56.09 mg/100 g.

3.2.2. *Orange (*Citrus *sp.)*

Citrus honeys are among the most appreciated in Morocco; citrus is a very light honey, with a heavy body and a delicious flavour. The free acidity ($\bar{x}=20.17$ meq/kg),

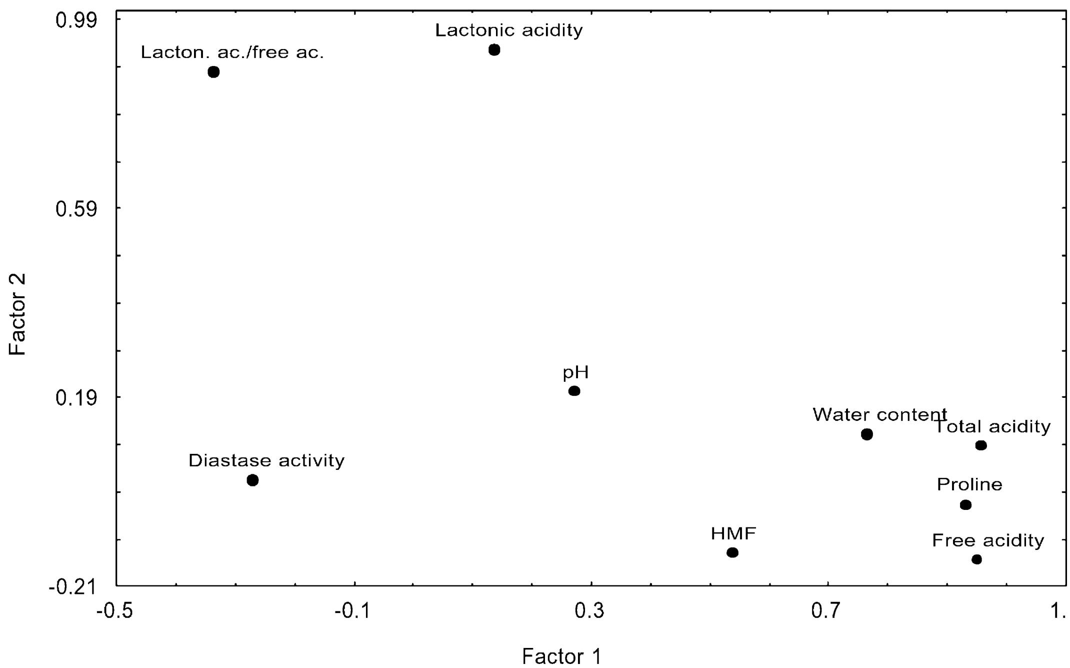

Fig. 1. Plot of factorial weights in first factor versus factorial weights in second factor from the principal component analysis of nine physicochemical parameters.

HMF ($\bar{x}$ = 17.7 mg/kg) and diastase activity ($\bar{x}$ = 40.1°Gothe) values are higher than those found by Peris (1981) in Spanish honeys and by Accorti, Persano, Piazza, and Sabatini (1986) in Italian honeys; however, the water content and pH showed sensitively low values ($\bar{x}$ = 16.8% and $\bar{x}$ = 3.55, respectively) when compared with those by these last authors. On the other hand, proline, with a mean value $\bar{x}$ = 25 mg/100 g, showed the lowest value when compared with the rest of the honey types.

*3.2.3. Loosestrife (*Lythrum *sp.)*

These honeys are poorly mentioned in the bibliography, characterised for their dark amber colour and strong flavour; the wax and cappings are golden yellow. This honey type shows median values for water content ($\bar{x}$ = 16.8%), pH ($\bar{x}$ = 3.62), free acidity ($\bar{x}$ = 28.4 meq/kg), proline ($\bar{x}$ = 38.5 mg/100 g) and diastase activity ($\bar{x}$ = 34.7°Gothe), and a medium-low value for HMF ($\bar{x}$ = 7.9 mg/kg).

*3.2.4. Apiaceae (*Ammi visnaga*,* Eryngium campestre*,* Ridolfia segetum*)*

These honeys are quite common in northern Morocco, due to the great extentions of field eryngo (*Eryngium campestre*), bishop's flower (*Ammi visnaga*), and false caraway *Ridolfia segetum*; they are characterised by their light amber colour. The values obtained for this honey type are quite similar to those for the previous one (loosestrife), but they can be differentiated by the medium–high proline content of the Apiaceae honeys ($\bar{x}$ = 63.9 mg /100 g).

*3.2.5. Honeydew honey (*Quercus *sp.,* Cedrus atlantica*)*

These are honeys with a great commercial interest. They are characterised by their very dark colour and strong flavour, and are heavy-bodied honeys; they are also slow to granulate. This is the only non-floral honey type in this study and is characterised by its very high values for water content ($\bar{x}$ = 20.3%), pH (4.28), free acidity (88.2 meq/kg), HMF (31.6 mg/kg) and proline ($\bar{x}$ = 227 mg/100 g), as well as the lowest value found for the diastase activity ($\bar{x}$ = 11.2°Gothe).

3.3. Statistical approach

To establish the differences between the five unifloral honey types found (*Eucalyptus*, *Citrus*, *Lythrum*, Apiaceae and honeydew), principal component analysis (PCA) and the stepwise discriminant analysis (SDA) statistical techniques were applied to the physicochemical data.

Table 2 shows the factor loading matrix obtained for the two factors and the variance explained by each of them. The first principal component accounts for 42.8% of the variance, and the second for 19.6%, the former being strongly chemically correlated with water content, free acidity, total acidity and proline, and the latter specifically with the lactonic acidity and lactonic acidity/free acidity. The cumulative variance is approximately 62%, which shows that the five honey types are not well distinguished by their physicochemical parameters. A scatter plot was obtained, correlating the factorial weights of features in the first factor against the factor-

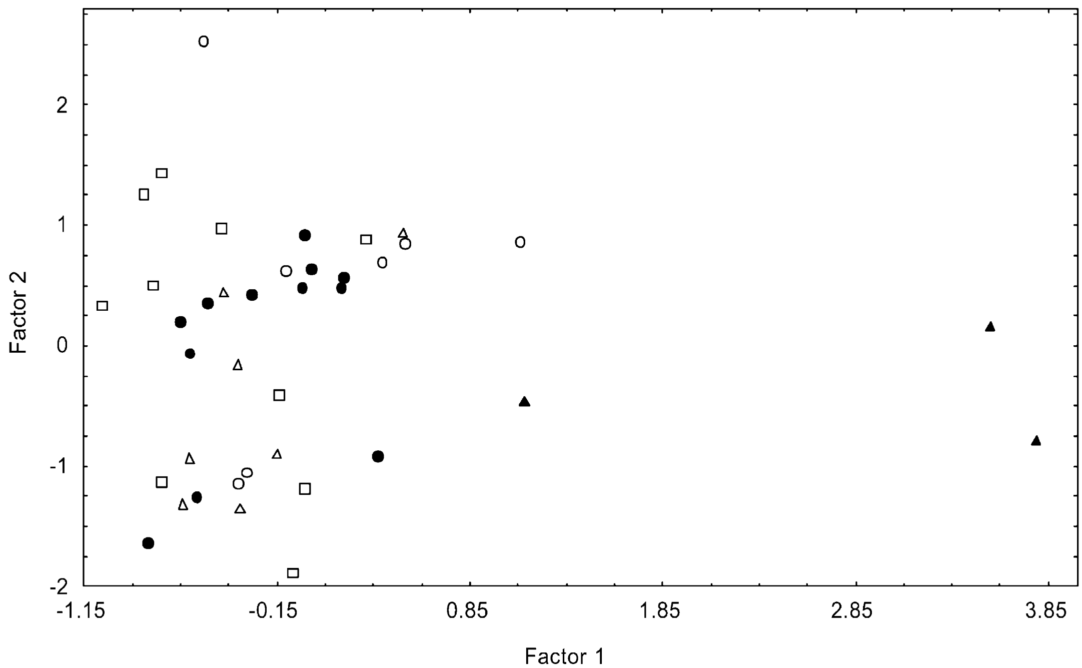

Fig. 2. Plot of the first factor versus second factor, for classification of the five unifloral honeys. (●): *Eucalyptus*; (□): *Citrus*; (○): *Lythrum*; (△): Apiaceae; (▲): Honeydew.

ial weights in the second factor. It can be seen from Fig. 1 that water content, free acidity, total acidity and proline are the dominant parameters in the first factor, while lactonic acidity and lactonic acidity/free acidity ratio dominate the second factor. Fig. 2 represents the graphic distribution of the samples according to their factor scores, and shows that only the honeydew honeys are perfectly differentiated from the rest, tending to higher values of the first component.

With respect to the discriminant analysis, a forward iterative inclusion of variables was performed in order to choose the parameters with a higher discriminant power. A tolerance of 0.01 eliminates the variables that provide superfluous information at a 99% level, along with those previously included in the model. The variables selected by stepwise discriminant analysis were, pH, free acidity, lactonic acidity/free acidity, HMF and proline, as well as the Wilks' lambda, which indicates the contribution of each variable to the discrimination; as can be seen, the latter does not surpass 0.09 (see Table 3). It is noteworthy that the significance level is high ($P<0.01$), except for the lactonic acidity/free acidity and the HMF. From this it can be concluded that the selected parameters have a relatively high discriminant power.

Table 4 lists the cumulative proportion of total dispersion, and the standardised coefficients for the five canonical variables. The higher the absolute value of a standardised coefficient, the more significant is the related selected variable in the canonical variable. pH appears to be the variable (standardised coefficients = 2.076), that accounts for most of the discrimination between honey classes (86%). The second canonical variable is closely related to pH and lactonic acidity/free acidity ratio (1.11 and −0.700, respectively), and explains more than 95% of the variance.

The general shape of the distribution of unifloral honeys scores, on a scatter diagram whose axes are the

Table 2
Rotated factor *loadings*, explained and cumulative variance

	Factor 1	Factor 2
pH	−0.2490	0.2288
Water content	−0.7492	0.1856
Free acidity	−0.9614	−0.0611
Lactonic acidity	−0.0480	0.9373
Total acidity	−0.9426	0.1796
Lactonic acidity/free acidity	0.4221	0.8440
HMF[a]	−0.5465	−0.0875
Diastase activity	0.2717	−0.0109
Proline	−0.9291	0.0532
Variance explained (%)	42.8012	19.6013
Cumulative variance (%)	42.8012	61.9901

[a] HMF, hydroxymethylfurfural.

Table 3
Results of stepwise discriminant analysis (SDA) of physicochemical parameters in some Moroccan unifloral honeys

Parameters	Wilks' λ	*F* statistic	*P* significance level
pH	0.074480	4.031366	0.009844
Free acidity	0.092806	6.868788	0.000475
Lactonic acidity/free acidity	0.054614	0.955626	0.445996
HMF[a]	0.065495	2.640332	0.053191
Proline	0.075467	4.184224	0.008245

[a] HMF, hydroxymethylfurfural.

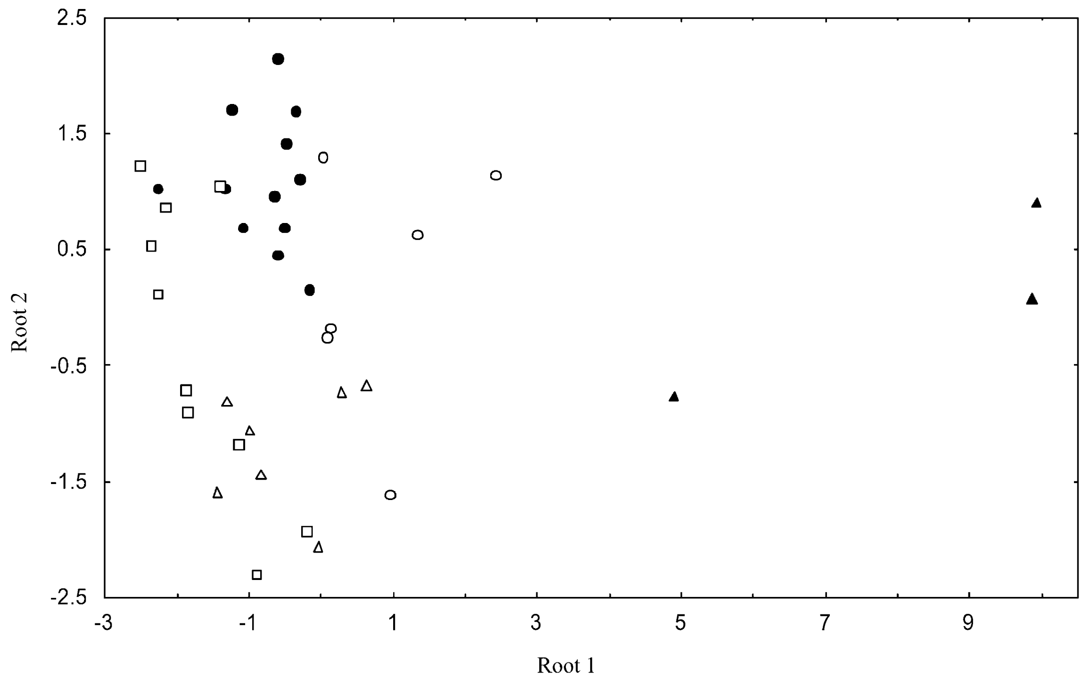

Fig. 3. Discriminant analysis of some Moroccan unifloral honeys, as shown by a scatter diagram representing the projections of the points of each unifloral honey, on the plane formed by the two principal canonical variables. (●): *Eucalyptus*; (□): *Citrus*; (○): *Lythrum*; (△): Apiaceae; (▲): Honeydew.

Table 4
Cumulative proportion of total dispersion, and standardised coefficients for canonical variables obtained by discriminant analysis of physicochemical parameters in some Moroccan unifloral honeys

Parameters	Canonical variable			
	1	2	3	4
pH	2.0765	1.10513	0.70310	1.45377
Free acidity	0.0698	−0.11020	−0.06526	0.02375
Lactonic acidity/free acidity	−0.0871	−0.70033	−1.91960	2.44627
HMF[a]	−0.0179	0.07146	−0.07983	−0.00866
Proline	0.0192	0.02836	0.02162	−0.00818
Cumulative proportion of total dispersion	0.8621	0.95188	0.98914	1.00000

[a] HMF, hydroxymethylfurfural.

Table 5
Coefficients for classification functions of some Moroccan unifloral honeys

Parameters	Honey type				
	Eucalyptus	*Lythrum*	*Citrus*	Apiaceae	Honeydew
pH	52.098	49.998	48.4296	55.124	69.269
Free acidity	0.439	0.687	0.6010	0.684	1.226
Lactonic acidity/free acidity	−3.742	−3.228	−0.2735	−2.162	−2.525
HMF[a]	0.542	0.355	0.5288	0.381	0.362
Proline	0.100	0.048	0.0211	0.096	0.230
Constant	−110.022	−103.943	−98.4590	−126.490	−236.954

[a] HMF, hydroxymethylfurfural.

Table 6
Classification matrix of some Moroccan unifloral honeys on the basis of physicochemical parameters

Honey type	% Correct	*Eucalyptus*	*Lythrum*	*Citrus*	Apiaceae	Honeydew
Eucalyptus	100	12	0	0	0	0
Lythrum	72	0	5	1	1	0
Citrus	70	1	2	7	0	0
Apiaceae	71	1	1	0	5	0
Honeydew	100	0	0	0	0	3
Total	82	14	8	8	6	3

first two canonical variables, is shown in Fig. 3, where a perfect separation of the honeydew honey from the rest can be observed.

Classification functions are linear combinations of the variables selected by the program; the coefficients and constants for these functions are shown on Table 5. By applying these functions to the samples, their validity can be verified, according to the agreement percentages of the cases in their corresponding group (see Table 6). It is seen that all *Eucalyptus* and the honeydew honey samples were correctly classified into their a priori established honey types (100%). *Citrus*, *Lythrum* and Apiaceae honeys show lower agreement percentages (<71%).

In view of the results obtained by PCA and SDA, it can be concluded that the physicochemical parameters analysed in this study are insufficient to achieve a perfect discrimination of the five unifloral honey classes considered, except for the eucalyptus and honeydew types.

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