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ORIGINAL ARTICLE

Characterisation of Moroccan Spurge (*Euphorbia*) honeys by their physicochemical characteristics, mineral contents and colour

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Euphorbia regis-jubae;
PCA;
SDA

Abstract The quality of 27 Moroccan Spurge (*Euphorbia*) honey samples was assessed. Eight physicochemical parameters and mineral composition were analysed and the CIELAB colour parameters (L^*, a^*, b^*, C^*_{ab} and h_{ab}) were determined. Results show no significant differences between the two *Euphorbia* honey types (*Euphorbia officinarum* subsp. *echinus* and *Euphorbia regis-jubae* honeys) regarding the physicochemical parameters. Sodium and magnesium show average values that can help to differentiate between *E. officinarum* subsp. *echinus* and *E. regis-jubae* honeys. Potassium was quantitatively the most important mineral (66% of the total minerals quantified), while sodium and calcium were present in moderate amounts (20% and 11% of the minerals, respectively). The colour parameters also have shown significant differences between *E. officinarum* subsp. *echinus* and *E. regis-jubae* honeys.

Considering the total information from physicochemical, mineral and colour data, Principal Component Analysis (PCA) and Stepwise Discriminant Analysis (SDA) were carried out to distinguish between the two *Euphorbia* honey types. PCA showed that the cumulative variance was approximately 56%. The results of SDA showed that parameters with a higher discriminant power

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were Na, Mg, L^*, C^*_{ab} and h_{ab}, and almost 100% of the samples were properly classified in their corresponding group, except for one sample.

1. Introduction

Honey is one of the most complex foodstuffs found in nature, and certainly the only sweetening agent that can be used by humans without processing. It is the sweet liquid produced by bees. They ingest nectar and honeydew, enrich it with substances of their own, store and mature it in honeycombs. Characterisation of unifloral commercial honeys is a hard task, initiated in Europe in response to the consumer's demand not only for a basic quality level but also for a certificate of geographical and botanical origin. This led to diverse EU regulations which state that, the country of origin where the honey has been harvested, and botanical origin of honeys must be shown on the labels (EU Council, 2002). Thus, the control of honey requires determining parameters that could unequivocally establish its origin, and calls for efforts to improve honey characterisation.

Melissopalynology consists of the analysis and identification of pollens contained in honey. It was the first technique used and, currently is complemented by sensory analysis, continues to be a reference tool for this purpose. However, its known limitations have encouraged the search for appropriate physicochemical parameters as honey indicators.

Monofloral honeys, originating predominantly from a single botanical source, are in higher demand from the consumer, which means that they also have a higher commercial value for the producers. Therefore, the characterisation of honeys is necessary in order to better our response to consumer demands (Pires et al., 2009; Feás et al., 2010; Estevinho et al., 2012; Iglesias et al., 2012). Some unifloral honeys have specific chemical or physical properties, which may be used to confirm the results of microscopical analysis. Methyl anthranilate or the flavonoid hesperetin was considered as an indicator of citrus honey (Serra Bonvehí and Ventura Coll, 1995); norisoprenoid *S*-dehydrovomifoliol for heather honey (Tan et al., 1989); honeydew honeys have a high electrical conductivity and contain much melezitose (Kirkwood et al., 1960), etc.

Morocco has a long beekeeping tradition. The number of hives and beekeepers is estimated to be approximately 375,000 and 35,000, respectively. The total of annual honey production is estimated at 3500 tons, of which eucalyptus, thyme, spurge, citrus and carob tree honey represent the greatest amount; however, the most preferred by consumers are thyme and spurge honeys. Despite its melliferous tradition, Morocco remains a country of importation of honey.

Researches on honey involving both palynological and physicochemical studies have been developed in Morocco in the last years. Thus, the characterisation of a number of unifloral honeys, such as eucalyptus, citrus, sunflower, crucifer, heather, mint and honeydew honeys has been carried out (Damblon, 1988; Terrab et al., 2002, 2003; Díez et al., 2004). However, although in some Mediterranean countries such as Lebanon, Turkey and Morocco the *Euphorbia* honeys are considered very valuable honeys from a consumers' point of view (Ricciardelli D'Albore, 1998), there are few studies on this honey type, being in fact one of the less studied. Melissopalynological studies on honey containing *Euphorbia* pollen are very scarce and only studies from the Canary Islands and Turkey can be found in the literature (La-Serna Ramos et al., 2001; La-Serna Ramos and Gómez Ferreras, 2006; Silici and Gökceoglub, 2007), and physicochemical characterisation of unifloral *Euphorbia* honeys is almost unknown (Chakir et al., 2011).

Euphorbia honeys are known for their anti-inflammatory, analgesic, and antibacterial capacity as well as their capacity to promote wound healing (Khiati et al., 2013). On the other hand, the intracellular latex-secretion and the extracellular nectar-secretion are anatomically connected in the spurge species. As the latex of *Euphorbia* species contains several toxic compounds, the secretion of these compounds into the nectar and finally into the honey may cause medical problems; thus, the toxicological and pharmacodynamic examinations of mixed honeys containing *Euphorbia* honey are recommended (Tóth-Soma et al., 1993).

Due to the large amount of *Euphorbia* (spurge) honey produced in the Ifni Massif region (SW of Morocco), its wide geographical distribution, the appreciation of this honey by the consumers, as well as the scientific and commercial importance of the characterisation of unifloral honeys, the main goal of the study included in this work was the physicochemical characterisation of two *Euphorbia* honeys produced in Morocco: *Euphorbia officinarum* subsp. *echinus* and *Euphorbia regis-jubae*. The study includes eight common physicochemical parameters (water content, pH, free, lactonic and total acidity, ash and electrical conductivity), the principal mineral elements (Na, Mg, Al, K, Ca and Fe), and the CIELAB colour characteristics (L^*, a^*, b^*, C^*_{ab} and h_{ab}).

2. Materials and methods

2.1. Honey samples

27 Spurge honey samples (11 samples from *E. officinarum* subsp. *echinus* and 16 from *E. regis-jubae*) were collected directly from professional beekeepers during 2009 and 2010 in the Ifni Massif region (SW Morocco). All samples were immediately transferred to the laboratory, and kept stored unpasteurised at 4–5 °C. These samples were the same as analysed by Terrab et al. (2014).

2.2. Pollen analysis

The botanical origin of the honeys was stated using the techniques described by Maurizio (1979) and Erdtman (1960). 10 g of honey was dissolved in 20 mL of diluted sulphuric acid (5 g H_2SO_4/L) and centrifuged (10 min at 2500 rpm); the supernatant was decanted and the sediment washed twice with 10 ml distilled water and again centrifuged. The sediment was extended on a slide and dried at 70 °C, and then mounted with stained glycerine gelatine. Following Behm et al. (1996), at least 500 pollen grains were counted among four different slides for each honey sample. For the pollen identification, the general key to pollen types from Díez (1987) was used.

2.3. Physicochemical common parameters

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

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

The free, lactonic and total acidities were determined by the titrimetric method: the addition of 0.05 M NaOH was stopped at pH 8.50 (free acidity), then 10 mL 0.05 M NaOH was immediately added and back-titration with 0.05 M HCl to pH 8.30 was carried out without delay (lactone acidity). Total acidity results were obtained by adding free plus lactone acidities (AOAC, 1990).

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

Electrical conductivity of a honey solution at 20% (dry matter basis) in CO_2−free deionised distilled water was measured at 20 °C (AOAC, 1990) in a Crison Basic 30 conductimeter.

Ash percentage was determined by calcination in furnace at 550 °C, until attaining constant mass (AOAC, 1990).

2.4. Determination of mineral elements

Mineral elements were determined using an Agilent 7500 c with Octopole Reaction System inductively coupled plasma-mass spectrometer. The analytical parameters for ICP-MS were: Nebulizer, Babington; RF generator, 1500; Argon flow rate, 1.11 L min^{-1}; Nebulizer pump, 0.1 rps; reduction gas flow, H_2 at 3.5 mL min^{-1} and He at 4 mL min^{-1}. The elements standards solutions were prepared by diluting stock solution (ICP standard CertiPUR) 1000 mg L^{-1}. The same procedure

Table 1 Distribution data for common physicochemical parameters in Moroccan *Euphorbia* honeys. *E. officinarum* subsp. *echinus* (samples 1–11) and *E. regis-jubae* (samples 12–27).

Sample	Moisture (%)	pH	Free acidity (meq kg^{-1})	Lactonic acidity (meq kg^{-1})	Total acidity (meq kg^{-1})	Electrical conductivity (μS cm^{-1})	Ash (%)	HMF (mg/kg)
1	19.60	3.95	53.22	2.49	55.71	525	0.14	2.54
2	20.00	3.80	50.97	3.24	54.21	497	0.13	3.89
3	17.30	4.00	46.39	1.50	47.89	586	0.30	20.96
4	19.30	3.93	39.84	3.00	42.84	437	0.10	10.93
5	18.00	3.80	38.55	1.50	40.05	444	0.16	9.73
6	18.70	3.78	80.28	3.48	83.76	677	0.04	51.65
7	17.10	4.32	39.72	2.60	42.32	874	0.07	7.18
8	21.70	3.90	44.30	2.70	47.00	517	0.17	4.64
9	16.00	4.67	34.00	3.35	37.35	860	0.40	3.99
10	17.60	4.11	33.90	4.05	37.95	529	0.24	11.22
11	18.20	3.94	16.05	0.50	16.55	227	0.04	85.48
Mean	18.50	4.02	43.38	2.58	45.97	561.18	0.16	19.29
SD	1.59	0.27	15.81	1.04	16.30	187.20	0.11	26.02
Range	16–21.70	3.78–4.67	16.05–80.28	0.50–4.05	16.55–83.76	227–874	0.04–0.40	2.54–85.48
12	19.20	3.76	40.61	14.40	55.01	370	0.24	0.90
13	17.50	3.81	37.30	14.00	51.30	454	0.07	10.18
14	21.00	3.81	37.46	14.10	51.56	570	0.13	3.74
15	20.00	3.85	54.75	16.65	71.40	570	0.08	25.92
16	18.30	3.87	29.10	14.90	44.00	380	0.11	7.93
17	19.90	3.79	33.71	13.70	47.41	553	0.17	3.44
18	18.10	3.85	32.46	13.85	46.31	569	0.03	0.75
19	16.40	3.91	38.09	14.90	52.99	528	0.21	3.59
20	19.80	3.82	47.59	2.70	50.29	661	0.16	3.15
21	17.80	3.75	38.82	2.60	41.42	679	0.11	4.48
22	18.90	3.74	45.52	4.30	49.82	567	0.22	4.46
23	19.40	3.77	45.56	2.20	47.76	650	0.16	1.79
24	19.80	3.76	52.00	5.50	57.50	690	0.22	2.25
25	16.40	3.76	27.27	4.00	31.27	322	0.17	5.49
26	15.80	3.73	38.82	3.80	42.62	563	0.17	2.24
27	18.00	3.69	46.67	0.55	47.22	685	0.22	1.94
Mean	18.52	3.79	40.36	8.88	49.24	550.69	0.15	5.14
SD	1.49	0.06	7.84	5.99	8.53	115.87	0.06	6.07
Range	15.80–21	3.69–3.91	27.27–54.75	0.55–16.65	31.27–71.40	322–690	0.03–0.24	0.75–25.92

was applied to prepare a Rh solution (1 mg L^{-1}) which was chosen as the internal standard. All reagents were analytical grade. All dilutions were prepared with deionised water produced by a Milli Q water purification system (Millipore, Belford, USA).

Six mineral elements (Na, Mg, Al, K, Ca and Fe) were determined in each honey. Samples were prepared from exactly 0.5 g put into PTFE vessels and dissolving with 6 mL 67% HNO_3 (PlasmaPURE, SCP, Courtaboeuf, France). The digestion was carried out in microwave oven (Multiwave 3000, Anton Paar, Austria), setting the parameters 10 min 0 to 800 W, 10 min 800 W, and 15 min vent.

2.5. Colour parameters

Colour was assessed by tristimulus colorimetry based on the reflectance spectra, with the application of the equations proposed by the *Commission Internationale de l'Éclairage* (CIE, 2004). The reflectance spectra were measured directly on the honey against a white background, with a CAS-140B spectroradiometer (Instrument System, Munich, Germany) equipped with a Top 100 telescopic probe and a Tamron SP 23A zoom (Tamron USA, Inc., Commack, NY, USA), set to measure in the visible region (λ = 380–770 nm) at constant intervals ($\Delta\lambda$ = 2 nm). Spectra were integrated using the CromaLab® software (Heredia et al., 2004), which takes into account the CIE recommendations. The uniform colour space CIE 1976-($L^*a^*b^*$) (CIELAB) was considered. As is required by the weighted-ordinate method ($\Delta\lambda$ = constant), the visible reflectance spectra of the samples were weighted according to the characteristic factor of the selected visual reference conditions: 10° visual field observer and the CIE standard illuminant D65, which correspond to natural daylight (Wyszecki and Stiles, 1982). The CIELAB colour parameters L^*, a^*, b^*, C^*_{ab} and h_{ab} were determined. L^* represents the perceived lightness, a^* and b^* indicate the change in hue from red to green and from yellow to blue, respectively. Chroma (C^*_{ab}) represents the amount of colour and is measured according to the distance to the origin of coordinates. It is the attribute that allows determining the difference in comparison to a grey colour with the same lightness for each hue, so it is considered to be the quantitative attribute of colourfulness. Hue (h_{ab}) is the attribute according to which colours have been traditionally defined as reddish, greenish, etc. This attribute is related to

Table 2 Distribution data for mineral content (mg/kg), and colour variables measured by diffuse reflectance method in the CIE 1976-L^* a^* b^* (CIELAB) colour space in Moroccan *Euphorbia* honeys. *E. officinarum* subsp. *echinus* (samples 1–11) and *E. regis-jubae* (samples 12–27).

Sample	Na	Mg	Al	K	Ca	Fe	L^*	a^*	b^*	C^*_{ab}	h_{ab}
1	160	35	19	770	370	6	43.43	30.54	32.26	44.42	46.56
2	170	26	19	670	121	6	39.00	29.19	25.87	39.00	41.55
3	48	19	16	920	51	4	41.55	31.50	30.30	43.71	43.89
4	37	25	18	550	114	5	46.97	31.92	39.67	50.92	51.18
5	192	22	6	500	90	5	50.68	29.22	44.39	53.15	56.64
6	198	28	19	980	90	7	38.55	27.38	24.80	36.94	42.16
7	19	65	18	1500	130	8	39.88	29.25	27.05	39.84	42.76
8	182	30	16	640	111	4	47.60	29.70	38.47	48.60	52.33
9	26	91	24	1660	140	8	40.78	26.64	27.39	38.21	45.80
10	190	23	20	800	160	6	45.60	30.80	36.94	48.10	50.18
11	175	22	11	160	96	2	43.97	28.95	34.64	45.14	50.11
Mean	127	35	17	832	134	5.5	43.46	29.55	32.89	44.37	47.56
SD	26.02	75.96	22.42	4.80	432.5	83.50	3.93	1.61	6.44	5.43	4.87
Range	19–198	19–91	6–24	160–1660	51–370	2–8	38.55–50.68	26.64–31.92	24.80–44.39	36.94–53.15	41.55–56.64
12	160	10	40	460	85	2	74.83	16.23	73.11	74.89	77.48
13	220	11	12	530	90	2	74.96	16.80	77.57	79.37	77.78
14	310	11	6	510	90	3	82.16	11.81	77.34	78.23	81.31
15	260	18	10	870	66	6	45.92	32.53	37.75	49.84	49.25
16	214	12	6	470	76	1	81.11	10.51	77.81	78.51	82.31
17	270	12	4	500	100	2	83.46	9.08	81.80	82.30	83.67
18	364	15	21	540	220	4	80.49	11.42	83.59	84.37	82.22
19	233	16	8	620	68	3	63.52	23.71	64.97	69.16	69.95
20	302	14	5	720	70	6	69.02	23.51	71.66	75.42	71.84
21	374	13	7	630	100	2	74.18	16.49	77.62	79.35	78.01
22	300	15	7	630	250	3	75.01	19.92	79.28	81.74	75.89
23	280	16	6	710	127	3	72.37	20.13	76.36	78.97	75.23
24	250	17	9	700	100	4	66.81	25.85	71.23	75.77	70.06
25	200	10	21	380	42	3	80.89	6.58	67.03	67.35	84.39
26	200	11	11	520	80	3	71.92	16.89	75.22	77.10	77.34
27	290	19	16	630	56	10	65.12	16.92	63.10	65.33	74.99
Mean	264	13.7	11.8	589	101	3.6	72.61	17.40	72.22	74.86	75.73
SD	59.18	2.91	9.17	123.3	56.21	2.19	9.41	6.81	10.85	8.51	8.38
Range	160–374	10–19	4–40	380–870	42–250	1–10	45.92–83.46	6.58–32.53	37.75–83.59	49.84–84.37	49.25–84.39

the differences in absorbance at different wavelengths and is considered the qualitative attribute of colour.

2.6. Statistical analysis

Multivariant statistical treatments (Principal Component Analysis, PCA, and Stepwise Discriminant Analysis, SDA) were carried out using the software Statistica v 8.0 (Statsoft Inc., 2007).

3. Results and discussion

3.1. Pollen analysis

The microscopical analysis of the sediment showed that *Euphorbia* honeys contained between 27% and 99% pollen of *Euphorbia* sp. For the *E. officinarum* subsp. *echinus* honey, the most characteristic accompanying species were *Eryngium ilycifolium*, *Bellis* sp., *Capsella* and *Reseda* sp. However, for the *E. regis-jubae* honeys, the most characteristic accompanying species were *Crepis Anthemis*, *Bellis*, *Capsella*, *Coronilla viminalis*, *Lotus* and *Limonium*. For more details see Terrab et al. (2014).

3.2. Physicochemical common parameters

Table 1 shows the means, standard deviations and ranges of the different physicochemical parameters data.

Water content, which is related to the climatic conditions, the season and the degree of maturity (White, 1975; Bogdanov et al., 1999), showed values between 16% and 20% in most of the samples (90%), although two samples exceeded the permitted limit established by the European Community Directive (EU Council, 2002). No differences were found between the two *Euphorbia* honey types (*E. officinarum* subsp. *echinus*, mean = 18.50 and *E. regis-jubae*, mean = 18.52). Terrab and Heredia (2004) found similar values of moisture in avocado honeys from S Spain and Canary Island, a region with quite similar climatic conditions to the Ifni Massif region. They also match with those values reported for honeys from another arid region, the Argentinean Patagonia (Aloisi, 2010; Aloisi et al., 2013).

The pH exerts great importance during the extraction and storage of honey since influences the texture, stability and shelf life. In this study, pH values ranged between 3.69 and 4.67, with mean values 4.02 and 3.79 for *E. officinarum* subsp. *echinus* honeys and *E. regis-jubae* honeys, respectively.

Acidity of honey is due to the presence of organic acids and inorganic ions such as gluconic acid with their lactones or esters, phosphate and chloride. Acid measurement appeared useful to assess honey fermentation, to authenticate unifloral honeys and to differentiate nectars from honeydew honeys (Nandaa et al., 2003). Concerning free acidity established by legislations, European Community regulation requires in general not more than 50 meq kg^{-1} and not more than 80 meq kg^{-1} for baker's honey (EU Council, 2002). Table 1 shows values ranging from 16 to 80 meq kg^{-1} being 85% of the samples below the EU limit of tolerance. These values were higher than those reported by Terrab et al. (2002) for four different unifloral Moroccan honeys. No differences were found regarding free acidity between *E. officinarum* subsp. *echinus* honeys (mean = 43.38) and *E. regis-jubae* honeys (mean = 40.36); nevertheless, the laconic acidity (considered as the acidity reverse when honey becomes alkaline) showed a slight difference between the two honey types.

The hydroxymethylfurfural (HMF) content is a freshness indicator, so it is an important criterion to evaluate storage

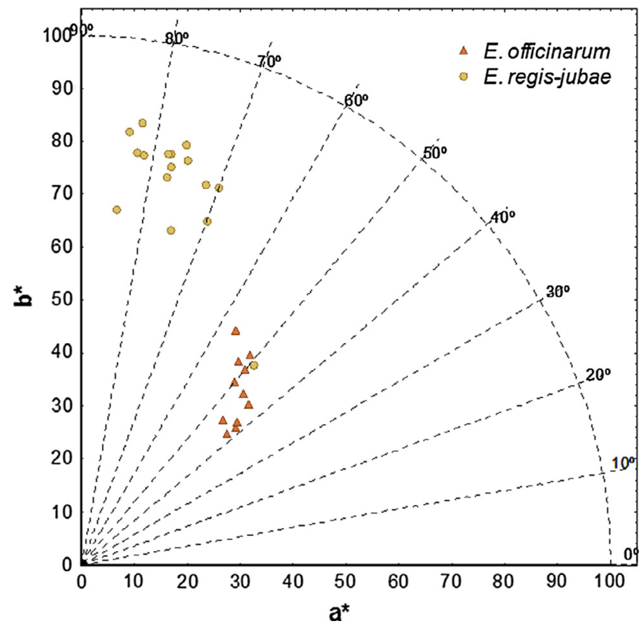

Figure 1 Distribution of the Moroccan *Euphorbia* honeys (*E. officinarum* subsp. *echinus* and *E. regis-jubae*) within the CIELAB colour space (a^*b^*-diagram).

Table 3 Rotated factor *loadings*, explained and cumulative variance.

	Factor 1	Factor 2
Moisture	−0.1288	0.6603
pH	0.7255	−0.5470
Free acidity	0.3278	0.7808
Lactonic acidity	−0.5104	0.0627
Total acidity	0.0782	0.7742
E.C.	0.4171	−0.0094
Ash	0.2336	−0.4848
HMF	0.2573	0.2224
Na	−0.7511	0.3184
Mg	0.8208	−0.3615
Al	0.3815	−0.1775
K	0.7580	−0.1580
Ca	0.1851	0.0699
Fe	0.7257	0.1314
L^*	−0.9376	−0.1941
a^*	0.8131	0.2992
b^*	−0.9320	−0.1437
C^*_{ab}	−0.9275	−0.1145
h_{ab}	−0.9340	−0.2216
Variance explained (%)	41.43	14.49
Cumulative variance (%)	41.43	55.93

E.C., Electrical conductivity.

time and heat damage (Ruoff and Bogdanov, 2004). The EU legislation (EU Council, 2002) establishes a maximum limit of 40 mg/kg for this parameter. Fresh honey does not contain, or contains only trace amounts of HMF (Bogdanov et al., 2004). From consumers' point of view, low HMF values guarantee that the honey is practically unaltered (Escriche et al., 2008). In this study, *Euphorbia* honey samples showed HMF values ranged from 2.54 to 85.48 mg/kg for *E. officinarum* subsp. *echinus* honeys, and from 0.75 to 25.92 mg/kg for *E. regis-jubae* honeys. Two samples were found to surpass the limit permitted by the European Community regulations.

The ash content is generally used to determine the botanical origin (floral, mixed or honeydew) of honeys (White, 1978). In this case, this parameter showed typical floral honeys values, and ranged between 0.04 and 0.4 for *E. officinarum* subsp. *echinus* honeys, and from 0.03 to 0.24 for *E. regis-jubae* honeys. None of the samples surpassed 0.6%, which is the permitted limit for flower honeys (EU Council, 2002). The mean value of ash found in this study was slightly lower than that found in *Eucalyptus* (0.22%) and Apiaceae (0.18%) Moroccan honeys, but higher than *Lythrum* (0.10%) and *Citrus* (0.07%) Moroccan honeys (Terrab et al., 2003).

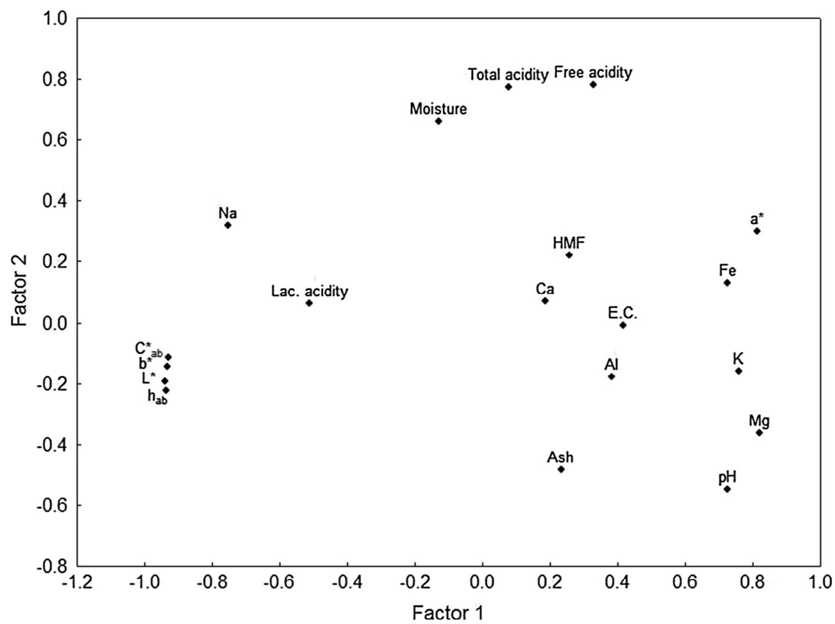

Figure 2 Plot of factorial weights. First factor versus second factor from the Principal Component Analysis of 19 physicochemical, minerals and colorimetric parameters.

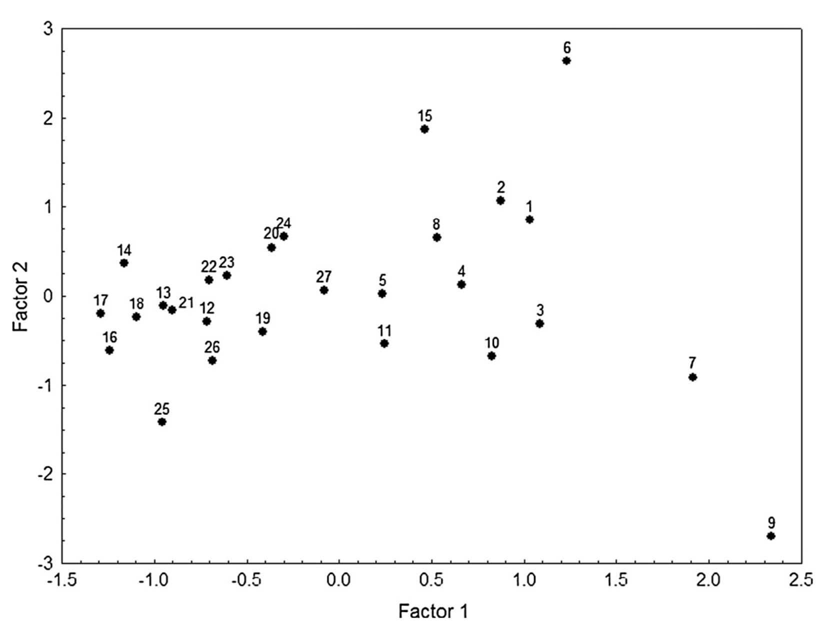

Figure 3 Plot of the first factor versus second factor for the classification of the two *Euphorbia* honey types. *E. officinarum* subsp. *echinus* (samples 1–11) and *E. regis-jubae* (samples 12–27).

The electrical conductivity of honeys is closely related to the concentration of mineral salts, organic acids and proteins. This parameter varies greatly according to the floral origin, so it is considered one of the best differentiating parameters to classify honeys regarding their floral origin (Krauze and Zalewski, 1991; Mateo and Bosch-Reig, 1998; Terrab et al., 2002). In this study the electrical conductivity showed values between 227 and 874 $\mu S\,cm^{-1}$ for *E. officinarum* subsp. *echinus* honeys, and between 322 and 690 $\mu S\,cm^{-1}$ for *E. regis-jubae* honeys. Based on these results and other from literature, the electrical conductivity could be considered a reliable parameter to differentiate our samples from other unifloral honeys such as citrus (136–474 $\mu S\,cm^{-1}$), rosemary (89–250 $\mu S\,cm^{-1}$), heather (815–1092 $\mu S\,cm^{-1}$), or honeydew honey (1329–2400 $\mu S\,cm^{-1}$) (Mateo and Bosch-Reig, 1998; Terrab et al., 2003).

3.3. Mineral elements

Mean contents of each mineral found in the 27 honeys expressed in mg/kg fresh weight are shown in Table 2. The potassium was quantitatively the most important mineral (66% of total minerals quantified), having an average content $\bar{x} = 687$ mg/kg; sodium and calcium were present in moderate amounts in the honeys (20% and 11% of total minerals, respectively), showing magnesium, aluminium and iron the lowest average contents (2%, 1.5% and less than 0.5% of total minerals, respectively). Sodium and magnesium showed average values that can help to differentiate between *E. officinarum* subsp. *echinus* (Na = 127 mg/kg; Mg = 35 mg/kg) and *E. regis-jubae* (Na = 264 mg/kg, Mg = 13.7 mg/kg) honeys.

3.4. Colour parameters

Table 2 shows the results obtained for the different colour parameters in the CIELAB colour space against white background. The lightness (L^*) values ranged between 38.55 and 50.68 units for *E. officinarum* subsp. *echinus* honeys (dark honeys), and between 45.92 and 83.46 units for *E. regis-jubae* honeys (light honey). The chroma (C^*_{ab}) showed significant differences between *E. officinarum* subsp. *echinus* (mean = 44.37 units) and *E. regis-jubae* honeys (mean = 74.86 units). The results showed greater differentiation regarding the hue (h_{ab}), the qualitative attribute of colour, between *E. officinarum* subsp. *echinus* (36.94–53.15°, amber honeys) and *E. regis-jubae* honeys (49.84–84.37°, yellowish honeys). This can be observed graphically in Figure 1 which shows the projection of the colour points corresponding to each honey sample on the (a^*, b^*)-plane. The samples were located in a wide range of hue angles and chroma values, being the *E. officinarum* subsp. *echinus* honey samples separated from *E. regis-jubae* honey samples, except for one sample of *E. regis-jubae* that was located within the E. *officinarum* subsp. *echinus* honey samples area.

3.5. Statistical approach

To distinguish between the two *Euphorbia* honey types (*E. officinarum* subsp. *echinus* and *E. regis-jubae* honeys), Principal Component Analysis (PCA) and Stepwise Discriminant Analysis (SDA) statistical techniques were applied to the data obtained from the common physicochemical parameters, the mineral composition, and the colour characteristics analysed.

Table 3 shows the factor loading matrix obtained for the two factors and the variance explained by each one. The first principal component accounted for 41.43% of the variance, and the second for 14.49%, the former being strongly chemically correlated with Na, Mg, K, L^*, a^*, b^*, C_{ab}, and h_{ab}, and the latter specifically with the free and the total acidity. The cumulative variance was about 56%. A scatter plot was obtained by correlating the factorial weights of features in the first factor against the factorial weights in the second factor. It can be seen from Figure 2 that Mg, a^*, K, Fe and pH are the dominant parameters in the first factor, while free acidity, total acidity and moisture dominate the second factor. Figure 3 represents the graphic distribution of the 27 samples according to their factor scores, and shows that *E. officinarum* subsp. *echinus* honey samples are perfectly differentiated from the *E. regis-jubae* honey samples, except for the one sample which was intermingled with the *E. regis-jubae* honeys.

With respect to the discriminant analysis, a forward iterative inclusion of variables was performed in order to choose the parameters with higher discriminant power. A tolerance

Table 4 Results of Stepwise Discriminant Analysis (SDA) of mineral and colour parameters in two unifloral *Euphorbia* honey types.

Parameters	Wilks' λ	F statistic	p signification level
Na	0.172167	1.398241	0.250234
Mg	0.161527	0.013926	0.907181
L^*	0.162631	0.157591	0.695390
C^*_{ab}	0.174504	1.702259	0.206111
h_{ab}	0.167832	0.834269	0.371409

Table 5 Cumulative proportion of total dispersion, and standardised coefficient for canonical variable obtained by discriminant analysis of mineral and colour parameters in two unifloral *Euphorbia* honey types.

	Canonical variable
Parameters	1
Na	0.302262
Mg	0.031256
L^*	−0.367547
C^*_{ab}	0.549782
h_{ab}	0.770736
Cumulative proportion of total dispersion	1.00000

Table 6 Classification matrix of two unifloral *Euphorbia* honey types on the basis of mineral and colour parameters.

Euphorbia honey type	% Correct	*E. officinarum* subsp. *echinus*	*E. regis jubae*
E. officinarum subsp. *echinus*	100	11	0
E. regis jubae	93.75	1	15
Total	96.29	12	15

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, Na, Mg, L^*, C_{ab} and h_{ab}, 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 0.175 (see Table 4). Table 5 lists the cumulative proportion of total dispersion, and the standardised coefficient for the canonical variable. The higher the absolute value of a standardised coefficient, the more significant is the related selected variable in the canonical variable. h_{ab} appeared to be the variable that accounts for most of the discrimination between the two *Euphorbia* honey types (standardised coefficient = 0.770). Classification functions are linear combinations of the variables selected by the programme. The coefficients and constants for these functions (data not shown) were applied to the honey samples; their validity can be verified according to the agreement percentages of the cases in their corresponding group (Table 6). It is seen that all *E. officinarum* subsp. *echinus* honey samples were correctly classified into their a priori established honey types (100%), and also, except for one sample, the *E. regis-jubae* honey samples were correctly classified.

4. Conclusions

The use of multivariate analysis is adequate to classify honey types from similar floral origin, as the case of *E. officinarum* subsp. *echinus* and *E. regis-jubae* honeys. It can be concluded that the mineral, physicochemical and colorimetric parameters analysed in this study are sufficient to achieve an excellent discrimination between the two unifloral honey classes considered.

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