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RESEARCH ARTICLE (Open Access)

Pollen spectrum and physicochemical attributes of sulla (*Hedysarum coronarium*) honeys of Médéa region (Algeria)

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Abstract:

The qualities of Sulla honey samples of Médéa region (Algeria) were evaluated by determing the pollen spectrum and physicochemical attributes. It is generally accepted that a minimum content of 45% of Hedysarum coronarium (Sulla) pollen is necessary to classify an Sulla honey as unifloral. The samples were analysed for parameters including moisture, electrical conductivity, hydroxymethylfurfural, pH, acidity (free, lactone and total), sugars (fructose, glucose, sucrose and total sugar), fructose+glucose, fructose/glucose, glucose/moisture, proteins and colour. Qualitative pollen analysis showed the presence of 76 types from 35 families, with Fabaceae, Cistaceae, Asteraceae, and Myrtaceae being the most frequent. The overall pollen content can be considered as medium. All the samples presented low values of hydroxymethylfurfural and lactone acidity. The degree of colour varied from white to amber. Moisture, proteins content, fructose, glucose, sucrose and electrical conductivity were according to international standards.

Keywords: pollen analysis; honey; physicochemical proprieties; sulla; Algeria.

1. Introduction

Honey is the natural, sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant sucking insects on the living parts of plants. Honeybees collect, transform and combine this with specific substances of their own, and then store it and leave it in the honey comb to ripen and mature [9].

Honey characteristics are the result of the combined influence exerted by several factors including: composition of local flora, flowering phenology, species selection by honeybee foragers and the timing of human operations for harvesting. Consequently, there is a strict link between the pollen types present in the honey and the plant species flowering in the foraging area [5].

Melissopalynology can be defined as a discipline that focuses on pollen collected by bees, is of great importance for honey quality control: the different pollen grains and honeydew elements are a good fingerprint for the environment where the honey comes from. Pollen analysis can therefore be useful in determining and controlling the honey's geographical and botanical origin [27].

The physicochemical parameters of natural honeys, such as moisture, diastase, sugars and HFM contents, acidity and specific conductivity, are strictly defined and constitute the quality indicators which characterise individual honey varieties [1, 17].

Sulla (Hedysarum coronarium L.) is a legume well adapted to semi-arid Mediterranean environments and represents an effective example of a multiple-use species exploited for environmental protection, landscape enhancement and honey production [22].

In Algeria was dominant pollen or secondary pollen in honeys from the northeast and centre of the country [7, 8, 14, 17].

There are many types of commercially available honey in Algeria, but consumers prefer some particular honeys more than others. They believe that a particular type of honey is superior to other types produced locally or imported from other countries around the world. For this, the characterisation of honeys is necessary in order to better our response to

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consumer demands Therefore, the present study was undertaken to characterise the pollen spectrum and physicochemical properties of sulla honey produced in Médéa region.

2. Material and Methods

2.1. Honey Samples

Twelve samples of sulla honeys (*Hedysarum coronarium*) produced in various regions of Médéa (Algeria) were collected from beekeepers in 2009. The samples were stored at 4–6°C until analyzed. All analyses were performed in triplicate. The regions from which the samples of honey were collected are indicated in Table 1.

Botanical origin of the honey samples were confirmed by the pollen analysis conducted according to Louveaux et al. [13].

2.2. Pollen Analysis

The pollen content of 12 honey samples produced by *Apis mellifera* was studied. For the quantitative and qualitative analysis, the method described by Louveaux et al. [13]. was followed. Under optical microscope the sediment of the honey sample (10 g) was analyzed.

Slides were prepared without acetolysis. The examination of the pollen slides were carried out with an optical microscope (400× or 1000×, as appropriate), in order to make sound identification of the pollen types.

About of 1000 grains of pollen per sample was counted. In order to recognize the pollen types, we used the reference collection of the Faculty of Sciences of Ourense (University of Vigo, Spain), reference pollens collected in the areas of the samples through spring-summer; different pollen morphology guides and information from different websites.

2.3. Physicochemical Analyses

Moisture was determined with a Carl-Zeiss Jena refractometer, by measuring the refractive indices at 20°C. The moisture content was calculated using the Wedmore table [4] and the results were expressed as percentages.

Electrical Conductivity was measured at 20°C in a 20% (w/v) honey solution (dry matter basis) in CO_2-free deionized distilled water [4] by a EUTECH instrument conductimeter (Con.520).

pH was measured by pH-meter (WTW inoLab pH 750) in a solution containing 10g of honey in 75 mL of distilled water [4].

Free, lactonic and total acidity were determined by the titrimetric method: the addition of 0.05 N NaOH, is stopped at pH 8.50 (free acidity), immediately a volume of 10 mL 0.05 N NaOH is added, and without delay, back-titrated with 0.05 M HCl from 10 mL to pH 8.30 (Lactonic acidity). Total acidity was obtained by adding free plus lactone acidities. Results were expressed as meq/kg [4].

Protein content was determined by the method of Azeredo et al. [5]. A volume of 0.1 ml of protein extract (honey sample 50% w/v) was added to 5 ml of Coomassie Brillant Blue. After 2 min of incubation, the quantity of proteins was estimated at 595 nm in relation to bovine serum albumin standard curve (10–100 µg/0.1 ml).

Hydroxymethylfurfural (HMF) was determined by HPLC according to Fallico et al. [10]. Aliquots of honey samples were diluted to 50 ml with distilled water, filtered on 0.45 µm filter (Albet, Barcelona, Spain) and injected into an HPLC system (Shimadzu Class VP LC-10ADvp) equipped with a diode array detector (Shimadzu SPD-M10Avp). The column was a Phenomenex Luna C18 (250 mm× 4.6 mm, 5 µm), fitted with a guard cartridge packed with the same stationary phase. The HPLC conditions were: isocratic mobile phase, 90% water at 1% of acetic acid and 10% methanol; flow rate, 0.7 ml/min; injection volume, 20 µl. All the solvents were of HPLC grade (Merck, Milan). The wavelength range was 220–660 nm and the chromatograms were monitorated at 285 nm. HMF in honey samples was identified by splitting the peak in honey with an HMF standard (Sigma-Aldrich, St. Louis, Mo., U.S.A.), and by comparison of the UV-spectra of the HMF standard with that of the honey samples. The amount of HMF was determined using a calibration curve of the HMF standard. Each sample was analyzed twice.

Fructose, glucose and sucrose were determined by HPLC method [12]. Aliquots of honey were diluted to 100 ml with distilled water, filtered through a 0.45-µm filter (Albet, Barcelona, Spain), and immediately injected into a Waters high-performance liquid chromatography (HPLC) constituted of a 600 Controller pump with a quaternary gradient pump system, a 717 plus Autosampler (Waters, Milford, MA, USA), a 410 Differential Refractometer refractive index (RI) detector. Separation of sugars was carried out using a Phenomenex PhenoSphere NH_2 80A column (250 mm×4.6 mm, 5 µm) fitted with

a guard cartridge packed with the same stationary phase. HPLC conditions were isocratic mobile phase, 80% acetonitrile and 20% water; flow rate, 1.8 ml/min; injection volume, 20 µl, with the column temperature maintained at 30°C.

Total sugars were determined using a special refractometer (Carl-Zeiss Jena refractometer) reading at 20 °C.

3. Results and Discussion

3.1. Pollen Analysis:

According the Maurizio's classification [13] of the quantitative analysis, 50% of the samples belonged to Class II (honey content: 20 000 –100 000 constituents per 10g), and 50% to Class III (honeys rich in pollen: 100 000 –500 000 constituents per 10g). The overall pollen content can be considered as medium (pollen density 2210– 34422 grains/g, average 13745 grains/g; Table 1).

Bees forage different plants; thus, honey is always a mixture of different sources [19]. Thirty five families and seventy six pollen types were identified in the whole samples (Table 2). In sulla honeys mean values for *Hedysarum* pollen were around 70%. The principal accompanying pollens were *Eucalyptus*, *Rosmarinus*, some Apiaceae, *Reseda*, *Salix* and *Sinapis* pollen type.

The Asteraceae and Fabaceae families provided the greatest number of pollen types with ten and seven pollen types each; respectively. The *Trifolium* pollen is present in all of the samples with a maximum value of 2.9% in sample M10. Next, the *Cistus* and Fabaceae pollen is present in 91.67% of the samples with a maximum value of 6.6% in the sample M20 and 3.9% in the sample M03 respectively. Seven pollen types (*Eryngium, Eucalyptus sp,* Apiaceae, *Daucus carota,* Brassicaceae, *Olea europaea* and *Reseda sp*) were found in more than 75% of samples and six pollen types (*Echium sp, Sinapis,* Rhamnaceae, *Salix sp, Genista* and Poaceae) were present in 50–66.67% of the samples.

3.1. Physicochemical Parameters

The means of physicochemical results detected in honey samples are given in Table 3.

The moisture content (%) in the investigated samples ranged from 13.96 to 18.16. All tested Sulla honeys had moisture contents below 20%, which is the maximum prescribed limit for the moisture content as per the Codex standard for honey [9].

The moisture content of honey is highly important factor contributing to its stability against fermentation and granulation during storage [21]. The different moisture content of honey depends on harvest season, the degree of maturity reached in the hive and moisture content of original plant [11].

Electrical conductivity (µS/cm) in honey samples varied in the range of 163–610. The conductivity of honey is the main quality parameter for this product; it is also important physicochemical measurement for the authentication of unifloral honeys [15]. According to Codex Alimentarius [9] the electrical conductivity (EC) value for the nectar honey should be less than 800 µS/cm (with few exceptions).

Table 1. Summarized results of the quantitative analysis

Samples	*Location*	*Harvested period*	*NGP*	*Maurizio class*
M01	Harbile	July 2009	20400	III
M03	Souagui	July 2009	9360	II
M06	Ouamri	July 2009	24000	III
M07	Moukorno	July 2009	9275	II
M08	Chreiguaia	July 2009	2750	II
M10	Zoubiria	May 2009	4000	II
M11	Tamesguida	July 2009	26000	III
M12	Temzguida	August 2009	12125	III
M13	Hamdania	June 2009	15875	III
M16	Ben chkaw	June 2009	34422	III
M17	Ouled bouachra	July 2009	4522	II
M20	Ain boussif	June 2009	2210	II

NPG: Number of pollen grains in 1 gram of honey.

Table 2. Results of the qualitative analysis, represented as percentages

		Samples											
Family	*Pollen type*	*M01*	*M03*	*M06*	*M07*	*M08*	*M10*	*M11*	*M12*	*M13*	*M16*	*M17*	*M20*
Apiaceae		-	+	1	-	4.5	2.9	2.9	+	3.7	-	2.5	1
	Ammi majus	+	-	-	-	+	-	-	-	+	-	-	+
	Conium Type	4.5	-	-	-	-	-	-	-	-	-	-	-
	Daucus carota	1.5	-	2.7	-	1.1	+	3.7	3.1	8.6	-	2.8	+
	Eryngium Type	-	+	5.4	1	1.6	2.4	+	+	+	-	+	+
	Pimpinella anisum	-	+	-	-	-	+	-	-	-	-	-	-
Asteraceae		-	-	+	-	+	-	-	-	+	-	+	-
	Achelia sp	1.2	-	-	-	-	-	-	-	-	-	-	-
	Ambrosia	+	-	-	-	-	-	-	-	-	-	-	-
	Bellis	-	-	-	-	-	-	+	+	-	+	-	-
	Carduus	-	+	+	-	+	-	-	+		-	+	-
	Carthamus	-	-	+	-	-	-	-	-	+	-	-	+
	Centaurea	-	-	-	-	-	+	-	-	+	+	-	+
	Chrysanthemum	-	+	-	-	-	+	-	+	-	-	-	+
	Echinops	-	-	+	-	+	+	-	-	-	-	-	
	Taraxacum Type	+	-	-	-	-	+	-	+	-	-	+	+
Betulaceae	*Corylus*	-	-	+	-	-	-	-	-	-	-	-	-
Boraginaceae	*Cerinthe major*	+	-		-	-	-	-	-	-	-	-	-
	Echium	1.6	+	+	-	+	-	1.9	-	1.2	+	+	-
Brassicaceae		1.6	1		+	-	-	+	+	+	+	+	+
	Brassica napus	-	-	2.4	-	-	1.1	-	-	+	3.7	-	-
	Matthiola tricuspidata	-	-	+	-	-	-	-	-	-	+	-	-
	Raphanus Type	-	-	-	-	-	-	+	+	-		-	-
	Sinapis Type	1.1	+	-	-	+	1,1	-	-	+	+	+	+
Casuarinaceae	*Casuarina*	-	+	-	-	-	-	-	-			1.7	+
Cistaceae	*Cistus*	+	2.1	+	+	-	1	+	+	+	+	+	6.6
	Halimium	-	-	-		-	-	-	-		-	-	+
	Helianthemum	-	-	-	1.4	-	-	-	-	+	-	-	-
Chenopodiaceae		-	-	-	-	-	-	-	-	+	-	-	-
	Chenopodium	-	-	-	-	-	-	+			+	-	-
Cyperaceae	*Carex*	+	-	-	-	-	-		+	-	-	-	-
Elaeagnaceae	*Elaeagnus angustifolia*	+	-	-	-	-	-	-	-	-	-	-	+
Euphorbiaceae		-	-	-	-	1.6	-	-	-	-	-	-	-
	Chrozophora tinctoria	-	-	-	-	+	+	-	-	-	-	-	-
	Euphorbia	+	-	-	-	-	-	-	-	-	-	-	-
Fabaceae		1.9	3.9	+	+	1.5	+	+	1.4	+	-	1.1	1.2
	Genista Type		4.9	+	+	-	-	+	2.4		-	-	+
	Hedysarum coronarium	**56.2**	**77.5**	**55.4**	**86.8**	**45.5**	**76.4**	**73.9**	**76.8**	**72.9**	**85.1**	**75.4**	**54.8**
	Lotus Type	-	-	-	-	6.2	-	-	2.9	-	2	2.6	-
	Onobrychis	+	+	-	-	-	-	+	-	-	-	-	+
	Ononis natrix	-	-	-	-	-	-	-	-	+	-	-	-
	Trifolium	2.7	+	1.6	+	+	2.9	+	2.6	+	1	1.9	1
	Vicia	+	-		+	-	-	-	-	-	-	-	-
Fagaceae	*Quercus*	+	+	-	-	-	+	-	-	-	-	+	-
Iridaceae		-	+	-	-	-	-	-	-	-	-	-	-
Lamiaceae	*Lavandula*	-	+	+	-	-	-	+	-	-	-	-	+
	Mentha	-	-	-	-	+	-	-	-	-	-	-	
	Rosmarinus officinalis	+	+	-	-	5.9	-	-	-	+	-	-	23.4
	Thymus	-	-	-	+	-	+	+	-	-	-	-	-
Liliaceae		+	-	-	-	+	-	+	-	-	-	2.7	-
Malvaceae	*Malva sylvestris*	-	-	-	-	-	-	-	-	-	-	-	+
Myrtaceae	*Eucalyptus*	12.6	2.3	14.1	2.5	1.5	4.9	+	3.1	1.2	-	+	
Oleaceae	*Olea europaea*		1.8	+		+	+	+	+	+		+	+

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Onagraceae	*Epilobium*	-	-	+	-	-	-	-	-	-	-	-	-
Arecaceae	*Chamaerops*	-	-	-	-	-	-	-	-	-	-	2.9	-
Plantaginaceae	*Plantago*	-	-	-	-	-	-	-	-	-	-	-	+
Papaveraceae	*Papaver rhoeas*	+	-	-	-	1.3	-	-	-	-	-	-	-
Poaceae		+	+	-	+	+	+	-	-	-	-	-	+
Rafflesiaceae	*Cytinus hypocistis*		-	-		-	+	-	-	-	-	-	2.0
Resedaceae	*Reseda*	1.1	+	-	2.2	-	+	3.2	+	4.5	4.3	-	1.1
Rhamnaceae		-	+	-	1.9	19.0	1.6	2.5	+	2.4	-	-	+
	Rhamnus Type	9.4	-	-	-	-	-	-	-	-	-	-	-
	Ziziphus lotus	-	-	10.4	-	-	-	-	-	-	-	-	-
Rosaceae	*Malus* Type	-	+	-	-	-	-	-	-	-	-	-	-
	Prunus Type	-	-	-	-	-	-	-	-	-	-	-	+
	Rosa	-	-	-	-	-	-	-	2.3	-	-	-	-
	Rubus	-	-	-	-	-	-	-	+	-	-	+	-
Rhutaceae	*Citrus*	-	-	-	-	-	-	+	-	-	-	2.9	-
Salicaceae		+	-	-	-	-	-	-	-	-	-	-	-
	Populus	-	-	-	-	-	-	-	-	-	-	-	+
	Salix	-	1.6	-	1.7	5.6	+	4.8	+	-	+	-	3.1
Scropholariaceae		-	+	-	-	-	+	-	-	-		-	-
Urticaceae		-	-	-	-	-	-	-	-	-	+	-	-
Ulmaceae	*Ulmus*	-	-	-	-	+	-	-	-	-		+	+
Vitaceae	*Vitis*	-	-	-	-	1.7	-	-	-	-		-	-
Zygophyllaceae	*Peganum harmala*	-	-	3.9	-	-	-	-	-	-	1.4	-	-

(+) Values below 1%; (-) absence of the pollen type.

This parameter depends on the ash, organic acids, proteins, some complex sugars and varies with botanical origin [23].

All the Sulla honeys analysed were found to be acidic in character. Their pH values ranged from 3.61 to 4.16. In general, honey is acidic in nature irrespective of its variable geographical origin. This parameter is of great importance during the extraction and storage of honey as it influences the texture, stability and shelf life of honey [24].

The acidity of honey is due to the presence of organic acids, particularly the gluconic acid, in equilibrium with their lactones or esters and inorganic ions such as phosphate and chloride [16]. The mean of total acidity (25.59 meq/kg) were within the allowed limits (below 40 meq/kg) indicating the absence of undesirable fermentations. Values for free acidity ranged from 16.04 to 27.12 meq/kg; the lactonic acidity (considered as the acidity reserve when the honeys become alkaline) ranged between 1.16 and 4.97 meq/kg. The results obtained are in agreement with reported data for honeys from other geographical locations [18].

The colour of the honey samples varied from a white (27 mm Pfund) to amber colour (92 mm Pfund). Honey colour is closely linked to botanical origin is used for honey classification. Generally, the colour is related to sensory properties such as flavour and odour. Several factors can influence honey colour such as floral source, mineral content and storage conditions [25].

In the current study the protein content (µg/g of honey) ranged from 763 to 1719, these values was comparable to that found in Brazilian honeys where it varied from 199 to 2236 µg/g [6]. The protein content in honeys can be attributed to the presence of enzymes, some of which are introduced by bees themselves, and others are thought to be derived from the nectar. The protein content of honey is normally less than 5 mg/g [3]. The level of protein is dependent on the type of flora and thus it is variable [20].

Hydroxymethylfurfural (HMF) content is used to evaluate the quality of honey. It is not generally present in fresh honey [10]. The HMF content in all honey samples was lower than the allowed maximum limit of 40 mg/kg recommended by Codex Alimentarius [9] and The Council of the European Union [26] . HMF values were very low (less than 15 mg/kg) indicated the high degree of freshness of studied honeys.

Total sugar ranged from 80.4 to 84.2%; fructose is always the most important sugar quantitatively followed by glucose. Our results show a mean glucose content of 30.05% and a mean fructose content of 42.02%. In this study, the combined level of these sugars (varied from 68.6% to 77.28%) is over 60 g/100 g of honey, in accordance with the European Community Directive [26], for all samples. The fructose and glucose content of any honey type

depends largely on nectar source (Anklam, 1998). Honey samples of different botanical origin had a wide range of fructose and glucose content. The average ratio of glucose/moisture (values ranged from 1.72 to 2.15) is a criterion for the prediction of granulation tendency. The fructose/glucose ratio was calculated for all samples. This ratio gives information about the crystallisation state of honey: when fructose is higher that glucose the honey is fluid [2].

Sucrose content (%) in the samples ranged from 0.51% to 7.37% (means 4.74%), an important sugar from the legislative point of view. The limit of sucrose content for *Hedysarum* honey allowed by the European Community Directive [26] is ≤10%. Studied honeys are authentic, because the obtained results complied with requirements of the international standards. The sucrose level can be increased if the beekeeper has over-fed the bees with sugar during the spring [3]. Moreover, a high content of this sugar means an early harvest of the honey [6].

Table 3. Some physicochemical characteristics of honey samples (Mean of three repetitions)

Parameter	*Samples*												
	M01	*M03*	*M06*	*M07*	*M08*	*M10*	*M11*	*M12*	*M13*	*M16*	*M17*	*M20*	*Means ± SD*
F (%)	43.23	42.80	42.27	40.83	43.97	37.48	43.76	43.12	44.43	41.55	39.20	41.65	42.02±2.05
G (%)	30.07	34.48	29.01	29.03	27.82	31.12	31.50	28.94	27.86	29.63	31.38	29.81	30.05±1.86
F+G (%)	73.30	77.28	71.29	69.87	71.79	68.60	75.26	72.06	72.29	71.17	70.58	71.46	72.08±2.33
F/G	1.44	1.24	1.46	1.41	1.58	1.20	1.39	1.49	1.59	1.40	1.25	1.40	1.40±0.12
G/M	1.83	2.15	2.08	1.99	1.96	2.08	1.97	1.72	1.73	1.81	1.73	1.90	1.91±0.15
TS (%)	81.9	82.2	84.2	83.6	84.0	83.3	82.2	81.5	82.2	81.9	80.4	82.6	82.48±1.12
Sucrose (%)	5.32	0.51	4.94	4.85	5.03	7.37	4.74	3.72	5.53	4.45	4.65	5.79	4.74±1.60
Moisture (%)	16.4	16.0	13.9	14.6	14.2	14.9	16.0	16.8	16.1	16.3	18.1	15.6	15.77±1.19
pH	4.05	3.85	4.14	3.71	4.16	3.76	3.88	3.70	3.82	3.61	3.67	4.06	3.87±0.19
ACL (meq/kg)	25.80	21.90	22.32	19.11	18.04	17.87	26.41	26.60	27.12	16.04	22.62	19.68	21.96±3.86
ACC (meq/kg)	2.98	3.14	3.49	3.30	3.16	3.66	4.63	4.64	4.41	1.16	4.97	4.05	3.63±1.03
ACT (meq/kg)	28.78	25.04	25.81	22.41	21.20	21.53	31.04	31.24	31.52	17.20	27.59	23.73	25.59±4.58
EC (µS/cm)	580	329	478	234	398	268	460	356	470	163	610	312	388.1±136.42
HMF mg/kg	6.24	2.78	3.64	7.82	4.84	1.70	10.51	10.92	11.88	10.35	7.03	2.42	6.68±3.64
Proteins µg/g	1633	983	1713	763	1090	767	1585	1104	1719	867	1018	1040	1190.2±367.2
Colour (mm Pfund)	92	71	92	35	62	41	83	71	83	27	55	83	66.25±22.39

FA: Free acidity, LA: Lactonic acidity, TA: Total acidity, EC: Electrical conductivity, F: Fructose, G: Glucose, TS: Total sugar, HMF: Hydroxymethylfurfural, G/M: glucose/moisture.

4. Conclusions

In conclusion, all the analyzed honey samples had excellent quality properties according to international standards. Algerian Sulla honeys are characterized by the presence of *Eucalyptus*, *Rosmarinus*, some Apiaceae, *Reseda*, *Salix* and *Sinapis* pollen type; by low levels of HMF and moisture and median values of proteins content. Further studies will take in consideration other honeys of different geographic and botanical origin in order to complete the Algerian honey characterisation.

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