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PHYSICOCHEMICAL AND POLLEN ANALYSIS OF LOCAL HONEYS FROM THE LIMA VALLEY (PORTUGAL)

M. L. Estevinho[a], X. Feás [b], Julio A. Seijas[b], María Pilar Vázquez-Tato[b]

[a]CIMO-Mountain Research Center. Agricultural College of Bragança, Polytechnic Institute of Bragança, Campus Santa Apolónia, Bragança, Portugal.

[b]Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Santiago de Compostela, Lugo, Spain

xesufeas@gmail.com

ABSTRACT

An evaluation was done on the physicochemical quality of nineteen (n=19) *Apis mellifera* honeys produced in the Lima Valley, Portugal. The rural landscape of the Lima Valley is valuable in social, environmental and economic terms and its conservation and preservation are essential. The evaluated physicochemical characteristics were: moisture, ash, electrical conductivity, pH, hydroxymethylfurfural (HMF), free acidity, apparent sucrose, reducing sugars and diastase activity. All honey samples met the International physicochemical quality standards. A short study of pollen source was done by acetolisis method. The families *Fabaceae* and *Rosaceae* were found with 6 and 2 pollen types each respectively. The most important pollen source is *Erica*, moreover two samples are listed as *Erica* monofloral honey.

KEYWORDS

Honey, Lima Valley, Portugal, quality, physicochemical characteristics, pollen analysis, *Erica*.

INTRODUCTION

Honey is one of the oldest sweetening agents and is defined as the natural substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of the plants or excretions of plant sucking insects on the living part of plants which honeybees collect, transform, and combine with specific substances of their own, store and leave in the honeycomb to ripen and mature [1]. The beneficial characteristics of honey are its high nutritional value and the fast absorption of its carbohydrates on consumption [2].

Bee honey is made up of water and sugars, with other minor components like: proteins, free amino acids, flavors, aromas, pigments, vitamins, and many volatile compounds. Variations in nectar content, together with other factors such as climatic conditions, soil type, beekeeper activities and such, contribute to the existence of different types of honeys. Differences in their composition, also mean differences in the organoleptic and nutritional properties of these honeys. Medical evidence of the importance of honey as a health food has not existed until recent times. Honey was found to be a suitable alternative for wound healing, burns and various skin conditions [3,4,5,6] and to potentially have a role within cancer care [7]. Thus, honey has retained its natural image and an increase in consumption can be attributed to the general increase in living standards and a higher interest in natural and beneficial health products [8,9] Therefore, the characterization of honeys is a hard task in response to consumer´s demands [10,11,12,13,14]. Standard Codex (SC) and European Union (EU) legislation are intended to establish the minimum marketing level of the product and the need for consumer protection through correct denominations.

The present study aimed to characterize, for the first time, honeys harvested in Lima Valley (Portugal), in respect to: (i) floral nectar origin and (ii) physico-chemical parameters (moisture, ash, pH, free acidity, electrical conductivity, hydroxymethylfurfural content, apparent sucrose, reducing sugars and diastase activity).

MATERIALS AND METHODS

Nineteen (n = 19) typical honey samples, from *Apis mellifera iberica*, were collected by beekeepers from separate hives found in the Lima Valley. This territory encompasses four counties with the following distribution, 5 samples from Viana do Castelo, 5 samples from Ponte de Lima, 6 samples from Ponte da Barca and 4 samples from Arcos de Valdevez. Figure 1 shows the pertinent identification of the honey sampling regions.

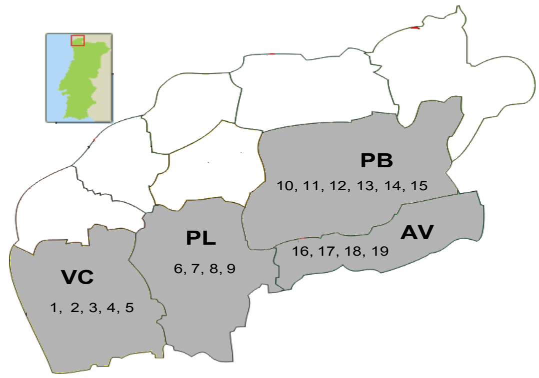

Figure 1. Map of Portugal Lima Valley showing honey sampling regions and distribution of the honey samples studied (n=19), VC (Viana do Castelo); PL (Ponte de Lima); PB (Ponte da Barca) and AV (Arcos de Valdevez).

The pollen spectrum of the honey samples was determined using the acetolytic method [15,16]. Physical and chemical analyses followed international recommendations [17,18]. In briefly:

-Moisture (M, %) was ascertained by refractometry, using an Abbe refractometer (Digital refractometer Atoga, Germany) and obtaining the corresponding % M (g/100 g honey) from the refractive index of the honey sample by reference to Chataway Charts [19].

-Electrical conductivity (EC, mS/cm) was measured in a Crison-522 conductimeter. Results were expressed as milliSiemens per centimeter (mS/cm).

-Ash (%), was obtained by calcination at 550 ºC, in a electric laboratory furnace SNOL 8.2/1100-1 (AB "Umega", Utena, Lithuania), to constant mass. Total ash contents, expressed as the percentage of residue left after dry oxidation by weight (g/100g honey).

-Free acidity (FA, meq/kg) was determined as by the titrimetric method to pH 8.50 by adding 0.05 N NaOH solution.

-pH values were measured using Digital pH Meter (pH-526 WTW Germany).

-Hydroxymethylfurfural (HMF, mg/kg) was determined after clarifying samples with Carrez reagents (I and II) and the addition of sodium sulfite. Absorbance was determined at 284 and 336 nm in a Perkin Elmer Luminescence Spectrophotometer (Norwalk, USA).

-Diastase activity (DA, Gothe degrees) was determined using a buffered solution of soluble starch and honey incubated in a thermostatic bath at 40 ºC until the endpoint was determined photometrically. The diastase number was calculated using the same time taken for the absorbance to reach 0.235, and the results were expressed in Gothe degrees as the amount (ml) of 1% starch hydrolyzed by an enzyme in 1 g of honey in 1 h.

-Reducing sugars (RS, %) were determined by reducing Soxhlet's modification of Fehling´s solution by titration using methylene blue as an internal indicator. The difference in concentrations of invert sugar before and after the hydrolysis procedure (inversion) was multiplied by 0.95 to reach the apparent sucrose (AS, %) content.

RESULTS AND DISCUSSION

Visually, all honey samples showed no sign of fermentation or granulation before the start of the analysis. Bees forage different plants; thus, honey is always a mixture of several sources. The economic and ecological importance of honeybee pollination is well-known [20]. However, in food control, pollen analysis is very efficient for the differentiation of honeys produced in distinctly different geographical areas [21,22]. Table 1 shows the results from pollen analysis of the honey samples.

	Honey Sample (Locality)						
Pollen type* (Family)	6 (PL[1])	7 (PL[1])	10 (PB[2])	11 (PB[2])	12 (PB[2])	16 (AV[3])	17 (AV[3])
Acacia (*Fabaceae*)	-	17	-	-	-	-	-
Castanea (Fabaceae)	-	-	11	8	-	-	-
Cistus (*Cistaceae*)	-	-	31	8	-	13	15
Echium (*Boraginaceae*)	20	6	-	8	-	-	-
Erica (*Ericaceae*)	20	39	-	26	59	25	50
Eucalyptus (*Myrtaceae*)	26	22	37	34	-	35	-
Genista (Fabaceae)	-	-	-	-	12	-	-
Medicago (*Fabaceae*)	7	-	-	-	6	-	-
Mentha (*Lamiaceae*)	-	-	-	6	-	-	-
Pinus (*Pinaceae*)	-	-	-	-	-	6	10
Prunus (*Rosaceae*)	20	11	-	-	11	-	-
Quercus (*Fagaceae*)	-	5	-	-	-	-	10
Rubus (*Rosaceae*)	7	5	-	8	6	25	15
Tilia (*Malvaceae*)	-	-	11	-	-	-	-
Trifolium (*Fabaceae*)	-	-	5	-	6	6	-
Vicia (*Fabaceae*)	-	-	-	8	-	-	-

Table 1. Pollen spectrum of studied honeys. [1]PL (Ponte de Lima); [2]PB (Ponte da Barca); [3]AV (Arcos de Valdevez).*Pollen type in percentages.

The families *Fabaceae* and *Rosaceae* were found with 6 and 2 pollen types each respectively. Generally speaking, monofloral honeys are made up of nectar belonging to a single plant in an extent of at least 45%. The results obtained from only two samples (12 and 17) gave monofloral *Erica* at 59% and a 50% of Erica pollen presence respectively. However, the technical requirements of the "*Terras Altas do Minho*" Portuguese honey, which is recognized as Protected Designation of Origin (PDO) honey in the EU, state that only 15% of *Erica sp*. pollen is necessary to declare the product under this protected denomination. From the total of samples analyzed, only sample 10 from Ponte da Barca don´t reach this value. Differences between North and South Portuguese honeys are possible, because of the absence of *Erica* pollen from the latter area [23]. *Acacia* pollen is present in one sample, and *Acacia* is known to be an invasive plant in Portugal [24]. Honey pollen analysis is a good parameter to measure the status of invasiveness of forest tree species outside their natural habitat.

Table 2 shows the results from physicochemical analysis of the honey samples. All of the values obtained for the physicochemical parameters fell within the maximum limits defined under current SC [1] and European legislation [25].

The M varied from 15.70 to 18.00 (mean value ± standard deviation = 16.87 ± 0.67%). In SC and EU Council directives, the maximum M content value of pure floral honey is given as not more than 20 % in general. The maximum amount of M present in honey is regulated for safety against fermentation, and is the only composition criteria, which as a part of the Honey Standard, has to be met for all world trade honeys. The water content of honey depends on various factors, for example the harvesting season, the degree of maturity reached in the hive and environmental factors [26]. The small variation observed in the water contents of these samples may be due to the similar bee-hive handling practices applied by Lima Valley beekeepers.

DA and HMF are parameters widely recognized for the evaluation of honey freshness and/or overheating. International regulations set a minimum value of 8 on Gothe's scale for diastase activity, and a maximum HMF content of 40 mg/kg. The HMF content of the honeys analyzed ranged from 2.50 to 18.20 mg/kg (mean value ± standard deviation = 10.01 ± 5.36 mg/kg). The HMF content is indicative of honey freshness [27] and from this point of view most of the analyzed samples are fresh, and thus, parallel the information provided by the producers. The DA of honey samples is 15.00 (average) with a range of 10.00 to 25.00 and a standard deviation of 3.60. Values obtained for HMF and DA are typical of unprocessed honey. In honey, these parameters are related to its quality and heat processing but have not been related to the origin of the samples [28]. No sample exceeded the limits established for these variables.

Ash values were below 0.60 %, as expected for nectar honeys. The honeys considered in this study had ash contents ranging from 0.88 to 0.41. The ash mass fraction is a useful parameter in determining botanical origin of honey and differentiating between nectar honey and honeydew.

The EC values of the honeys analyzed ranged from 0.64 to 0.72 mS/cm (mean value ± standard deviation = 0.68 ± 0.04 mS/cm). The EC of honey may be explained by taking into account the ash and acid content of honey, which reflects the presence of ions and organic acids; the higher their content, the higher the resulting conductivity.

The honey samples presented a pH from 3.56 to 4.70, with an average of 4.06. The low pH of honey inhibits the presence and growth of microorganisms and makes honey compatible with many food products in terms of pH and acidity. This parameter is of great importance during the extraction and storage of honey as it influences the texture, stability and shelf life of honey [29]. Published reports indicate that pH should be between 3.2 and 4.5 [30,17]. The values of pH in honey help to determine its origin, flower or forest; the latter show higher values.

	VC	PL	PB	AV	Total
Moisture	16.58±0.41 (15.90-17.00)	16.45±0.68 (15.70-17.20)	17.27±0.67 (16.50-18.00)	17.05±0.72 (16.40-18.00)	16.87±0.67 (15.70-18.00)
Electrical Conductivity	0.55±0.12 (0.42-0.73)	0.52±0.14 (0.33-0.67)	0.71±0.18 (0.45-0.90)	0.68±0.04 (0.64-0.72)	0.62±0.15 (0.33-0.90)
Ash	0.29±0.05 (0.22-0.36)	0.25±0.07 (0.18-0.33)	0.33±0.07 (0.22-0.41)	0.28±0.03 (0.26-0.31)	0.29±0.06 (0.18-0.41)
HMF	13.32±2.66 (9.90-16.30)	10.40±5.39 (5.20-17.80)	7.30±5.42 (2.50-16.80)	9.53±7.19 (2.80-18.20)	10.01±5.36 (2.50-18.20)
Diastase Activity	15.34±3.31 (10.70-20.00)	16.75±5.56 (13.00-25.00)	13.93±2.18 (12.00-16.60)	14.43±4.15 (10.00-19.00)	15.00±3.60 (10.00-25.00)
pH	4.04±0.43 (3.57-4.70)	3.96±0.30 (3.74-4.40)	4.08±0.36 (3.56-4.53)	4.16±0.39 (3.79-4.53)	4.06±0.35 (3.56-4.70)
Free Acidity	28.46±7.29 (19.90-37.00)	25.33±2.54 (22.30-28.50)	26.68±5.55 (19.80-33.20)	27.40±1.72 (25.70-29.80)	27.02±4.82 (19.80-37.00)
Reducing Sugars	74.08±5.59 (68.96-80.00)	71.96±1.35 (70.40-73.53)	75.28±7.64 (66.67-84.03)	68.21±3.91 (62.52-71.43)	72.77±5.78 (62.52-84.03)
Apparent Sucrose	4.64±0.37 (4.01-4.90)	3.84±0.59 (3.14-4.36)	3.52±1.15 (1.30-4.63)	4.14±0.26 (3.88-4.40)	4.01±0.82 (1.30-4.90)

Table 2. Physico-chemical parameters, moisture, ash, pH, free acidity, electrical conductivity, hydroxymethylfurfural (HMF) content, apparent sucrose, reducing sugars and diastase activity; of analyzed honey samples. mean±SD (range) PL (Ponte de Lima); PB (Ponte da Barca); AV (Arcos de Valdevez). Moisture, %; electrical conductivity, mS/cm; ash, %; hydroxymethylfurfural (HMF) mg/kg; diastase activity, Goethe degrees; free acidity, meq/kg; reducing sugars, % and apparent sucrose in %.

The FA of honey samples is 27.02 meq/kg (average) with a range of 19.80 to 37.00 and a standard deviation of 4.82 meq/kg. Variation in FA among different honeys can be attributed to floral origin [31] or to variation because of the harvest season [11]. The free acidity of honey may be explained by taking into account the presence of organic acids in equilibrium with their corresponding lactones, or internal esters, and some inorganic ions, such as phosphate [32]. All of the investigated samples met the demands imposed by the regulations, which require that the acidity should not exceed 40 meq/kg; this indicates the absence of unwanted fermentations.

Honey is mainly composed of the monosaccharides glucose and fructose. The RS content in the honeys analyzed ranged from 62.52 to 84.03 % (mean value ± standard deviation = 72.77 ± 5.78) and the mean percentages of AS 4.01 % showed a range of 1.30 to 4.90 and a standard deviation of 0.82 (sucrose content by European Directives must be under 5%). These two parameters confirm that the honey samples studied were floral honeys.

In 1992, the European Union (EU) created a system known as PDO to promote and protect names of quality agricultural products and foodstuffs [33]. Currently, in the EU, Portugal has the highest number of honeys, a total of nine, bearing the PDO logo; they are produced, processed and prepared in a given geographical area using certified know-how. To confirm our preliminary results, a larger sample data set is also in preparation with the aim of defining a possible new honey with a PDO denomination from this area of Portugal. Portuguese apiculture has been practiced traditionally by professional and semi-professional producers, many of whom migrate with their hives in order to take advantage of the different flowering periods [34]. The rural landscape of the Lima Valley has been highly praised in social and economic terms and its conservation and preservation are essential. The entire territory of the Lima Valley was included in the Zone of Action of European LEADER program. Most of the actions implemented were in the area of rural tourism-related activities [35]. Since their objectives are, among others, to preserve the farming systems based on the valorization of the natural environment and landscape, the agri-environmental activities and measures seem to be a viable means of reducing the abandonment of agriculture and depopulation [36]. Research, such as the study at hand, oriented toward assessment of floral origin and physico-chemical properties and provision of technical assistance to producers of Lima Valley honey may increase the commercial value of these products.

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