Research Article

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Pollen spectrum and physico-chemical attributes of heather (*Erica* sp.) honeys of north Portugal

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Abstract

BACKGROUND: Honey legislation has been addressed to establish the minimum marketing level of the product and the need for consumer protection through correct denominations. Research oriented toward assessment of floral origin and physico-chemical properties may increase the commercial value of these products. The characteristics of 23 unifloral honeys of *Erica* sp., from Portugal, were studied. Pollen features and some physicochemical parameters (moisture, ash, pH, free acidity, electrical conductivity, hydroxymethylfurfural content, apparent sucrose, reducing sugars and diastase activity) were determined.

RESULTS: All honey samples can be classified as monofloral *Erica* sp., they gave a mean value of 56% of *Erica* pollen type. The families *Fabaceae* and *Rosaceae* provided the greatest number of pollen types with 8 and 4 pollen types each respectively. The second most important pollen type is *Eucalyptus*, present in 69.6% of the samples. All honey samples met the international physicochemical quality standards. The present study found a linear correlation ($R = 0.996$) between the ash content of honeys and their specific conductivity.

CONCLUSION: All honey samples can be classified as monofloral *Erica* sp. Unifloral honeys are increasingly requested and appreciated, despite their higher prices. The samples were found to meet all major international honey specifications.

Keywords: honey; melissopalynology; physico-chemical analysis; *Erica* sp

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.[1]

Bee honey is made up of water and sugars, with other minor components such as proteins, free amino acids, flavours, aromas, pigments, vitamins and many volatile compounds. Variations in nectar content, together with other factors such as climatic conditions, soil type, and beekeeper activities contribute to the existence of different types of honey.[2] Differences in their composition cause differences in the organoleptic and nutritional properties of these honeys.[3]

The beneficial characteristics of honey are its high nutritional value and the fast absorption of its carbohydrates on consumption.[4] Furthermore, honey has a number of properties that are believed to facilitate the healing process. Honey was found to be a suitable alternative for healing wounds, burns and various skin conditions[5–9] and also to have a potential role in cancer care.[10]

The major consumers and importers of honey are the industrialised countries. An increase in consumption over the last few years can be attributed to the general increase in living standards and a higher interest in natural and beneficial health products.[11] Honey is a highly valuable ingredient in sauces, dressings, condiments, beverages and sweet and sour manufactured foods.[12]

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.[13–15]

Organoleptical properties, physico-chemical attributes and pollen spectrum are the main criteria for honey classification.[2] It is

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comparatively simple to measure them and they provide a good information value. The physico-chemical parameters of natural honeys, such as moisture, diastase, sugars and hydroxymethylfurfural (HMF) contents, acidity and specific conductivity, are strictly defined and constitute the quality indicators which characterise individual honey varieties.[16–19] The identification and quantification of pollen grains in honey sediment is the reference method used to determine the botanical origin of honey sample honeys. Usually, honeys are nominated as monofloral when at least 45% of pollen grain comes from the plant considered. For honey samples having under-represented pollen grains, (i.e. *Rosmarinus, Citrus, Thymus, Arbutus* and *Lavandula*), botanical classification may be achieved with a pollen frequency percentage of only 10–20%. However, for honey samples having over-represented pollen grains, (i.e. *Eucalyptus, Castanea, Cynoglossum* and *Myosotis*) botanical origin may be achieved with a pollen frequency percentage of 70–90%.

Portuguese apiculture has been practised traditionally by professional and semi-professional producers, many of whom migrate with their hives in order to take advantage of the different flowering periods.[20] In 1992, the European Union (EU) created a system known as Protected Designation of Origin (PDO), to promote and protect names of quality agricultural products and foodstuffs.[21] Currently, in the EU, Portugal has the highest number of honeys bearing the PDO logo, which are produced, processed and prepared in a given geographical area using certified expertise, namely: 'Mel da Serra de Lousã', 'Mel do Parque de Montezinho', 'Mel do Ribatejo Norte', 'Mel das Terras Altas do Minho', 'Mel da Terra Quente', 'Mel da Serra de Monchique', 'Mel do Alentejo', 'Mel dos Açores' and 'Mel de Barroso'.[22]

Heather honey is produced in Portugal from *Erica* sp., while in Spain and France it comes from either *Calluna* or *Erica* sp. This honey is characterised by its dark brown colour, strong flavour and a slightly salty taste. Consumers in Portugal prefer heather honeys and they are generally more costly than others.[23,24]

The purpose of this study was to investigate some properties of various honey samples collected from the north region of Portugal by using different honey analysis tests such as moisture, ash, pH, free acidity, electrical conductivity, hydroxymethylfurfural (HMF) content, apparent sucrose, reducing sugars and diastase activity. The determination of the frequency of pollen grains classes, were also determined in these honey samples, in order to verify the monofloral *Erica* sp. origin and to obtain a complete pollen spectrum.

MATERIALS AND METHODS

Honey sampling

Twenty-three typical honey samples, from *Apis mellifera*, were collected by beekeepers from different apiaries. The samples were from 14 localities of six districts in north Portugal. Figure 1 shows the geographical origin and identification code of the honey samples studied. All honey samples showed no sign of fermentation or granulation. They were obtained by centrifugation and stored at 5 °C until analysis, which occurred no more than 1 month after the extraction from the hives by beekeepers.

Sample floral-type identification

Even though the beekeepers themselves, according to the best of their knowledge and the location of hives, declared honey as monofloral heather honey, all the samples were subjected to qualitative pollen analysis as per Erdtman's acetolysis method.[25] The aim of that analysis was to confirm that analysed samples could be declared as heather monofloral honey.

Briefly, pollen analyses are based on the extraction of pollen grains from 10 g of crude honey. The sample was dissolved in distilled water and the sediment is concentrated by repeated centrifuging. About 10 mL of acetolysis mixture (Ac_2O/H_2SO_4, 9 : 1) is added and the tubes are incubated in a water bath (100 °C for 3 min), stirred vigorously, then centrifuged and decanted. About 12 mL of water-free acetic acid is added, stirred thoroughly, centrifuged, and decanted. The precipitate is washed in about 12 mL of distilled water, centrifuged, and decanted. 12 mL of 7% KOH is added, stirred thoroughly, centrifuged and decanted.

After this, pollen grains were stained with a solution of basic fuchsin and mixed with glycerin. The examination of the pollen slides were carried out with an optical microscope at ×400 and ×1000 in order to make sound identification of the pollen types. A minimum of 1000 pollen grains was counted per sample. In order to recognise the pollen types, we used the reference collection of the University of Santiago de Compostela's Pharmacy Faculty, different pollen morphology guides, and information from different websites.

Physicochemical analysis

Physicochemical parameters were analysed using the *Official Methods of Analysis* of the Association of Official Analytical Chemists (AOAC)[26] and The Harmonised Methods of the European Honey Commission.[27] Samples were analysed using the same methods during the same time period to ensure uniform conditions and comparability.

Moisture

The determination of water contents (moisture) were ascertained by refractometry, using an Abbe refractometer (Digital refractometer, Atoga, Germany). The refractometer was calibrated with distilled water before use. All measurements were performed at 20 °C. After waiting for 6 min for equilibration, and with the refractive index obtained, the conversion to the corresponding % moisture (g water 100 g^{-1} honey) by means of the Chataway table was carried out.[28]

Ash content

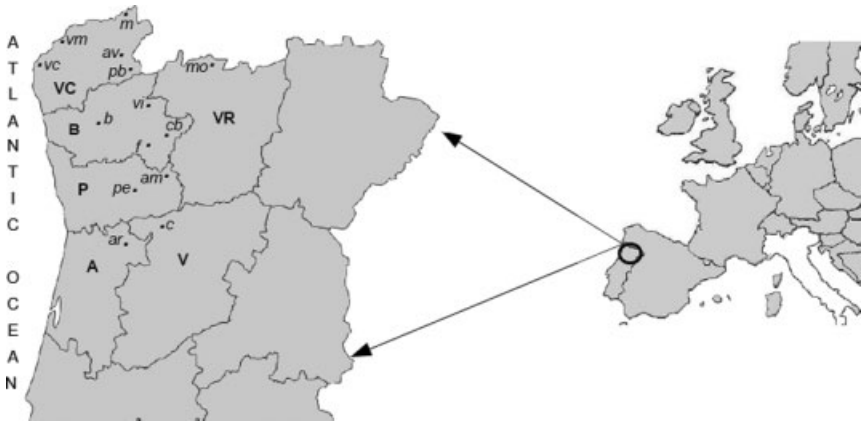
The ash content was determined by sample calcination at 550 °C, in a electric laboratory furnace SNOL 8.2/1100-1 (AB 'Umega', Utena, Lithuania) and calculated from the equation

$$\text{ash}(\%) = \frac{m_1 - m_2}{m_0} \times 100 \quad (1)$$

where m_1 is the mass of dish and ash, m_2 the mass of platinum dish prior to calcination and m_0 is the mass of the honey taken.

Electrical conductivity

Electrical conductivity of a honey solution at 20% (w/v) (dry matter basis) in CO_2-free deionised distilled water was measured at 20 °C in a Crison 522 conductimeter Crison, from Barcelona (Catalunya, Spain). Results were expressed as millisiemens per centimetre (mS cm^{-1}).



District	Locality	Identification
Aveiro (A)	Arouca (ar)	1, 2, 3
Braga (B)	Braga (b)	12
	Cabeceiras de Basto (cb)	10, 11
	Fafe (f)	7, 8, 9
	Vieira do Minho (vi)	4, 5, 6
Porto (P)	Amarante (am)	13, 14
	Penafiel (pe)	15
Viseu (V)	Cinfaes (c)	22
Viana do Castelo (VC)	Arcos de Valdevez (av)	20
	Melgaço (m)	16
	Ponte da Barca (pb)	19
	Vila Nova de Cerveira (vc)	17, 18
	Valença do Minho (vm)	21
Vila real (VR)	Montalegre (mo)	23

Figure 1. Map of Portugal showing honey sampling regions and distribution of the honey samples studied ($n = 23$).

pH and free acidity

Five grams of honey samples were diluted with 20 mL of distilled water and mixed thoroughly. The pH values for these samples were measured using a Digital pH Meter (pH-526 WTW, Weilheim, Germany). Free acidity was determined as follows by the titrimetric method: 10 g honey samples were dissolved in 75 mL of CO_2-free water in a 250 mL beaker. The electrode of the pH meter was immersed in the solution, stirred with a magnetic stirrer and titrated to pH 8.50 by adding 0.05 mol L^{-1} NaOH solution.

Determination of hydroxymethylfurfural

Hydroxymethylfurfural was determined by the standard method. In brief, 5 g of each honey sample were transferred to a 50 mL volumetric flask with a total of 25 mL of distilled water. After clarifying samples with 500 µL of Carrez reagents (I and II), samples were diluted to 100 mL with water. If necessary, alcohol may be added to suppress surface foam. With a clarified honey solution containing 0.2% (w/v) sodium bisulfite as a reference and a similar solution without bisulfite as a sample, a difference spectrum was obtained which represented only the HMF in the sample, without the interfering absorption of the honey. Absorbance was determined at 284 and 336 nm in a 1 cm quartz cuvette in a Perkin Elmer Luminescence Spectrophotometer (Norwalk, CT, USA).

HMF contents, expressed as mg kg^{-1}, were calculated from the equation

$$\text{HMF} = (A_{284} - A_{236}) \times F \quad (2)$$

where A_{284} and A_{236} are the absorbance readings, and F (mg kg^{-1}) is 149.7 was calculated with the equation

$$F = \frac{126 \times 1000 \times 1000}{16\,830 \times 10 \times 5} \quad (3)$$

where 126 is the molecular weight of HMF; 16 830 is the molar absorptivity of HMF at 284 nm; 1000 = mg g^{-1}; 10 = cL L^{-1}; 1000 = g kg^{-1} and 5 = g of honey.

Diastase activity

Diastase activity was determined using a buffered solution of soluble starch and honey incubated in a thermostatic bath at 40 °C. Thereafter, a 1 mL aliquot was removed at 5-min intervals and the absorption of the sample was followed at 660 nm in a Perkin Elmer luminescence spectrophotometer. 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 hydrolysed by an enzyme in 1 g of honey in 1 h.

Reducing sugars and apparent sucrose

Reducing sugars were determined by reducing Soxhlet's modification of Fehling's solution by titration at boiling point against a solution of reducing sugars in honey 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 content.

RESULTS AND DISCUSSION

Pollen analysis

The microscopic examination confirmed the identity of the honey source indicated by the beekeepers. All honey samples can be classified as monofloral *Erica* sp. Figure 2 shows the total pollen types identified and the % of *Erica* sp. pollen in each of the samples. Monofloral status generally refers to the presence of a single pollen type in quantities greater than 45% of the total pollen content in the spectrum. The number of *Erica* sp. pollen per sample varies between 45% (sample 15) and 71% (sample 3), the mean value being 56% with a standard deviation of 9%. The Portuguese heather honeys analysed have between five and eight pollen types, the mean number being 5.8. As we can see in Fig. 2, sample 12 showed eight pollen types, the highest value for all honey samples, and a 50% *Erica* pollen content.

Table 1 shows the frequency of occurrence of the 22 pollen types identified in the 23 samples. The *Fabaceae* and *Rosaceae* families provided the greatest number of pollen types with eight and four pollen types each, respectively. The second most important pollen type is *Rubus*, present in 73.91% of the samples with a maximum value of 26% in sample 22 and minimum value of 4% in samples 6 and 12. Next, the *Eucalyptus* pollen is present in 69.6% of the samples with a maximum value of 25% in sample 6 and minimum value of 6% in sample 18. Trifolium pollen is present in 60.86% of the samples. Bees forage different plants; thus, honey is always a mixture of different sources. However, in food control, pollen analysis is very efficient for the differentiation of honeys produced in distinctly different geographical and climatic areas.[2]

Physico-chemical parameters

Table 2 shows the results obtained from physico-chemical analysis of the honey samples. The moisture content (%) varied from 17.00 to 18.10 (mean value $\pm$ standard deviation $=$ 17.59 $\pm$ 0.37%). In *Codex Alimentarius* Standard and EU Council directives,[1,29] the maximum water content value of pure floral honey is given as 23% for heather (*Calluna*) honeys and not more than 20% in general. The maximum amount of water 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. Furthermore, the water content is also of great importance because it is considered to be a useful parameter for describing moistness and viscosity of honey. 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.[30] The small variation observed in

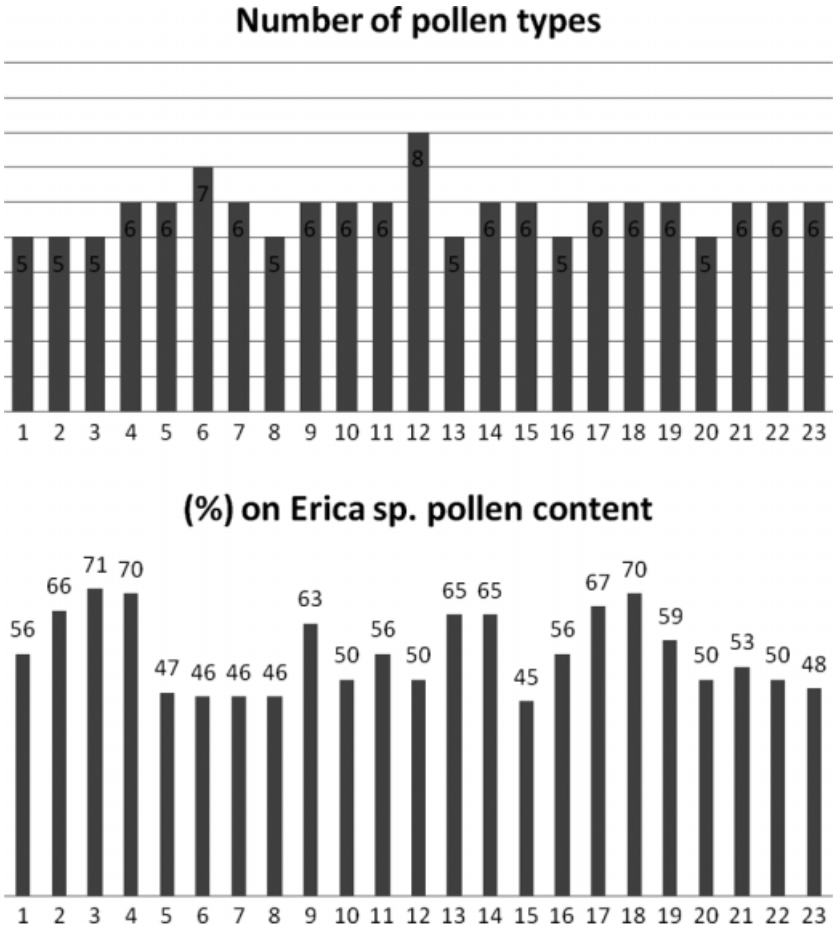


Figure 2. Graphs of the total number of pollen types and % of *Erica* sp. pollen in honey samples.

Table 1. Frequency classes and percentages of the pollen types in the honeys

		Honey sample																						
Family	Pollen type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Fabaceae	Acacia	ND	ND	ND	ND	ND	ND	i 12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Cytisus	ND	ND	ND	i 6	ND	i 13	ND	ND	ND	ND	ND	ND	ND	ND	ND	s 16	ND	ND	ND	ND	ND	ND	ND
	Chamaespartium	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	i 6	ND	ND	ND	ND	ND	ND	ND
	Genista	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	s 16	ND	ND	i 12	ND	s 16	ND	ND
	Lotus	ND	ND	ND	ND	i 5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Medicago	ND	ND	ND	ND	i 5	ND	ND	ND	i 4	ND	ND	ND	ND	ND	ND	ND	ND	ND	i 6	ND	ND	i 8	ND
	Trifolium	i 7	i 7	i 8	i 6	s 16	i 4	i 6	i 15	i 11	ND	ND	ND	i 6	i 7	ND	ND	i 5	ND	i 6	ND	ND	i 8	ND
	Vicia	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	i 7	ND	ND	8	ND	ND	ND	ND	ND	5	ND	ND
Rosaceae	Malus	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	s 24
	Prunus	ND	ND	ND	ND	ND	i 4	ND	ND	ND	i 11	i 4	ND	ND	ND	ND	ND	ND	ND	i 11	ND	ND	ND	m 2
	Pyrus	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	i 6	ND	ND	ND	ND	ND
	Rubus	I 7	i 7	ND	i 6	i 11	i 4	i 12	ND	ND	s 16	i 8	i 4	i 6	i 7	i 8	ND	i 5	i 6	i 6	i 15	ND	s 26	ND
Brasicaceae	Brassica	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	i 12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Rutaceae	Citrus	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	i 6	ND	ND	ND	ND	m 2
Cistaceae	Cistus	i 12	ND	i 4	ND	ND	ND	i 6	ND	i 4	i 6	i 4	ND	ND	i 7	i 8	ND	i 5	ND	ND	i 15	ND	ND	ND
Boraginaceae	Echium	ND	i 7	i 4	i 6	s 16	i 4	ND	ND	i 4	i 6	i 4	i 4	ND	ND	ND	ND	i 9	ND	ND	ND	ND	i 8	ND
Ericaceae	Erica	p 56	p 66	p 71	p 70	p 47	p 46	p 46	p 46	p 63	p 50	p 56	p 50	p 65	p 65	p 45	p 56	p 67	p 70	p 59	p 50	p 53	p 50	p 48
Myrtaceae	Eucalyptus	s 18	i 13	i 13	ND	ND	s 25	s 18	s 23	i 14	i 11	s 24	i 12	s 17	i 7	s 23	ND	ND	i 6	ND	ND	s 16	ND	s 22
Labiatae	LavaNDula	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	i 7	i 8	ND	ND	ND	ND	ND	ND	ND	ND
Pinaceae	Pinus	ND	ND	ND	i 6	ND	ND	ND	i 8	ND	ND	ND	i 4	ND	ND	ND	ND	ND	i 6	ND	i 10	i 5	ND	m 2
Fagaceae	Quercus	ND	ND	ND	ND	ND	ND	ND	i 8	ND	ND	ND	i 7	i 6	ND	ND	i 6	i 9	ND	ND	i 10	i 5	ND	ND
Asteraceae	Taraxacum	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	i 8	ND

p, predominant pollen (at least 45%); s, secondary pollen (16–44%); i, important minor pollen (3–15%); m, minor pollen (1–3%); ND, not detected.

Table 2. Physico-chemical parameters (moisture. ash. pH. free acidity. electrical conductivity. hydroxymethylfurfural content. apparent sucrose. reducing sugars and diastase activity) of analysed honey samples

Honey sample	Moisture (%)	Electrical conductivity (mS cm^{-1})	Ash (%)	Hydroxymethylfurfural (mg kg^{-1})	Diastase activity (Gothe scale)	pH	Free acidity (meq kg^{-1})	Reducing sugars (%)	Apparent sucrose (%)
1	18.1	0.79	0.35	16.3	25.0	3.73	39.2	74.07	3.85
2	17.5	0.79	0.35	22.5	20.0	3.74	39.3	73.53	3.22
3	17.5	0.69	0.29	7.1	15.0	3.88	25.7	71.43	4.11
4	18.1	0.70	0.30	2.8	15.0	3.88	33.4	71.43	3.04
5	17.5	0.75	0.32	0.9	11.5	4.07	31.7	72.99	3.73
6	17.5	0.67	0.28	4.4	20.0	3.77	31.9	74.07	3.85
7	17.2	0.52	0.20	1.7	13.64	3.92	21.9	75.76	3.42
8	17.2	0.64	0.26	1.9	12.0	4.09	25.3	76.92	3.53
9	18.1	0.67	0.28	1.8	20.0	4.03	30.2	73.53	3.79
10	17.2	0.67	0.28	8.4	20.0	4.01	27.1	68.49	4.27
11	17.1	0.61	0.25	1.4	25.0	3.90	27.4	69.93	4.46
12	17.5	0.94	0.43	13.6	20.0	4.24	34.0	70.42	4.00
13	17.5	0.73	0.31	2.3	12.0	3.93	31.1	72.99	4.30
14	17.8	0.75	0.32	2.5	13.0	3.99	29.8	74.07	4.44
15	18.0	0.61	0.25	22.8	23.0	3.47	45.2	72.46	4.24
16	17.2	0.70	0.30	9.2	12.0	3.70	36.9	74.07	3.85
17	17.2	0.73	0.31	1.7	20.0	4.05	23.5	74.07	3.85
18	17.3	0.73	0.31	0.9	15.0	3.94	26.7	72.99	3.73
19	18.1	0.76	0.33	6.2	30.0	4.03	33.2	66.67	3.56
20	18.0	0.64	0.26	2.8	10.0	3.79	29.8	69.44	3.88
21	17.0	0.70	0.30	5.8	25.0	3.94	24.2	69.93	3.42
22	18.1	0.66	0.27	18.5	20.0	4.08	27.8	68.96	4.34
23	17.8	0.85	0.38	6.6	14.0	3.82	35.1	71.43	3.57
Mean	17.59	0.71	0.30	7	17.87	3.91	30.89	72.16	3.85
DS	0.37	0.08	0.05	6.8	5.3	0.16	5.58	2.43	0.38
Maximum	18.10	0.94	0.43	22.8	30.0	4.24	45.20	76.92	4.46
Minimum	17.00	0.52	0.20	0.9	10.0	3.47	21.90	66.67	3.04

the water contents of these samples may be due to the similar beehive handling practices applied by Portuguese beekeepers. Many national beekeeping organisations have moisture content maximum values of 17.5–18.5% for special classes of quality honey.[31] The samples analysed in the present work reach this quality parameter.

Ash values were below 0.60%, as expected for nectar honeys (EU and Codex Standards).[1,22] The honeys considered in this study had ash contents ranging from 0.20 to 0.43. The ash mass fraction is a useful parameter in determining botanical origin of honey and differentiating between nectar honey and honeydew.

The electrical conductivity values of the honeys analysed ranged from 0.52 to 0.94 mS cm^{-1} (mean value ± standard deviation = 0.71 ± 0.08 mS cm^{-1}). The electrical conductivity 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 relation between electrical conductivity and ash content has been demonstrated by many researchers, who have been determined that the above-mentioned parameters are related.[32–34] The present study found a linear correlation ($R = 0.996$) between the specific conductivity of honeys and their ash content. The final regression model ($y = ax + b$), as presented in Fig. 3, between the ash fraction and electrical conductivity ($y = 1.806x + 0.164$) differs from the one proposed by the International Honey Commission (IHC): $y = 1.74x + 0.14$, where y is the electrical conductivity in mS cm^{-1} and ash is the ash mass fraction in g per 100 g.[27] The slope differs, but the section part of the relation formula does not vary as much. As an illustration, if a sample of Portuguese heather honey has an electrical conductivity of 1, the ash mash fraction is 0.46 g per 100 g as calculated by the obtained model, or 0.49 g per 100 g using the model proposed by the IHC. However, no statistically significant differences between models for different types of honey have been found in previous work.[33] The linear regression model of ash mass fraction and electrical conductivity is therefore independent of honey type.

Diastase activity and hydroxymethylfurfural (HMF) are parameters widely recognised 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^{-1}.[1,29] The HMF content of the honeys analysed ranged from 0.9 to 22.8 mg kg^{-1} (mean value ± standard deviation = 7.0 ± 6.8 mg kg^{-1}). The HMF content is indicative of honey freshness,[35] and from this point of view most of the analysed samples are fresh, and thus, parallel the information provided by the producers. The diastase activity of honey samples is 17.87 (Gothe degrees) (average) with a range of 10.00–30.00 and a standard deviation of 5.30 (Gothe degrees). In honey, HMF content is related to its quality and heat processing. Furthermore diastase activity has been related to the origin of the samples.[36,37] No sample exceeded the limits established for these variables. Considering that honey samples were collected during the same period, differences in HMF could be attributed to the variation in climatic conditions in the area.

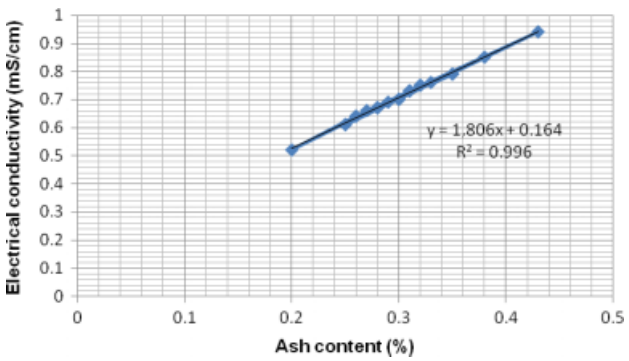


Figure 3. Linear regression between ash content and electrical conductivity in 23 *Erica* sp. honey samples from Portugal.

The honey samples presented a pH from 3.47 to 4.24, with an average of 3.91. The low pH of honey inhibits the presence and growth of micro-organisms 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.[38] Published reports indicate that pH should be between 3.2 and 4.5.[17,31] The values of pH in honey help to determine its origin: flower or forest; the latter shows higher values.

The free acidity of honey samples is 30.98 meq kg^{-1} (average) with a range of 21.90–45.20 and a standard deviation of 5.58 meq kg^{-1}. Variation in free acidity among different honeys can be attributed to floral origin[39] or to variation because of the harvest season.[14] 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.[40] All of the investigated samples met the demands imposed by the regulations, which require in general not more than 50 meq kg^{-1} and not more than 80 meq kg^{-1} (baker's honey).[29]

Honey is mainly composed of the monosaccharides glucose and fructose. The content (%) of reducing sugars in the honeys analysed ranged from 66.77 to 76.92% (mean value ± standard deviation = 72.10 ± 2.43) and the mean percentages of apparent sucrose (%) is 3.85% with a range of 3.04–4.46 and a standard deviation of 0.38 (sucrose content by European Directives must be under 5%). These two parameters confirm that the honey samples studied were floral honeys and had a good maturation grade.

Table 3 shows the physico-chemical parameters reported in literature for heather honey samples. As can be seen, the values recorded were similar to those obtained for heather honeys collected in Portugal[23], Spain[41] and Europe in general.[37]

CONCLUSIONS

In this work, melissopalinological analysis and the principal physicochemical parameters have been determined to characterise monofloral heather (*Erica* sp.) honey from north Portugal. All honey samples can be classified as monofloral *Erica* sp; they gave a mean value of 56% of *Erica* pollen type. The families *Fabaceae* and *Rosaceae* families provided the greatest number of pollen types with eight and four pollen types each respectively. An overall consideration of the samples analysed shows that the most important nectariferous taxa for the area studied are *Rubus, Eucalyptus* and *Trifolium* pollen types, present in 73.91%, 69.6% and 60.86%, respectively. All of the values obtained for the physico-chemical parameters analysed in this work fell within the maximum limits defined under current Standard Codex and European legislation. The present study found a linear correlation ($R = 0.996$) between the specific conductivity of honeys and their ash content. The regression model between the ash fraction and electrical conductivity was $y = 1.806x + 0.164$. From the economical standpoint, the assessment of a monofloral origin may increase the commercial value of these honeys.

Table 3. Physico-chemical parameters reported in literature for heather honey samples

Reference	Origin	Number of samples	Moisture (%)	Electrical conductivity (mS cm^{-1})	Ash (%)	HMF(mg kg^{-1})	Diastase activity (Gothe Scale)	pH	Free acidity (meq kg^{-1})	Reducing sugars (%)	Apparent sucrose (%)
23*	Portugal	20	17.31 (15.12–18.92)	5.44 (2.90–7.41)	0.36 (0.15–0.52)	20.4 (6.79–36.3)	21.8 (13.0–33.3)	4.01 (3.60–4.35)	30.5 (23.2–37.0)	72.1 (67.3–78.0)	1.41 (0.40–2.36)
23*	Portugal	20	17.32 (16.16–18.71)	5.02 (3.56–6.52)	0.32 (0.20–045)	15.3 (0.32–32.9)	23.9 (15.0–51.1)	4.21 (3.85–4.46)	23.4 (15.8–34.0)	71.7 (68.8–74.6)	0.96 (0.27–1.81)
23*	Portugal	20	18.86 (14.60–19.90)	5.23 (4.12–6.39)	0.34 (0.25–0.44)	12.0 (4.40–34.1)	25.8 (13.0–41.0)	4.13 (3.86–4.31)	31.0 (18.3–38.8)	73.0 (69.9–75.4)	1.00 (0.27–1.70)
41	Spain	11	18.19 (16.64–19.60)	ND	0.47 (0.35–0.59)	3.72 (0–20.19)	43.40 (30.30–75.0)	ND	35.66 (28.21–41.94)	65.86 (60.39–71.69)	1.80 (0.21–4.15)
37	Europe	219	18.5 (15.6–21.4)	0.73 (0.49–0.97)	ND	ND	23.4 (12.0–36.0)	4.2 (3.9–4.7)	32.1 (20.8–43.0)	73.4 (67.2–79.5)	1.4 (0.1–3.6)
PW	Portugal	23	17.59 (17.00–18.10)	0.71 (0.52–0.94)	0.30 (0.20–0.43)	7 (0.9–22.8)	17.87 (10.0–30.0)	3.91 (3.47–4.24)	30.89 (21.90–45.20)	72.16 (66.67–76.92)	3.85 (3.04–4.46)

Results are given as the mean (min–max).
* 'Serra da Lousã' honey from three consecutive harvest.
ND, no data.
PW, present work.

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