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Original article

Palynological and physicochemical data characterisation of honeys produced in the *Entre-Douro e Minho* region of Portugal

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Summary 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 physicochemical properties may increase the commercial value of these products. The characteristics of thirty-one honeys produced in the *Entre-Douro e Minho* region in Portugal were studied. Pollen features and some physicochemical parameters (moisture, ash, pH, free acidity, electrical conductivity, hydroxymethylfurfural contain, apparent sucrose, reducing sugars and diastase activity) were determined. The samples were found to meet international honey specifications. The present study found a linear regression between the ash content of honeys and their specific conductivity. Five samples are listed as *Eucalyptus* honey, one sample as *Citrus* honey, and twenty-five samples as multifloral honeys. Of the total, 87.1% exceeded the quality parameters and should be labelled as 'virgin' honey.

Keywords *Entre-Douro e Minho,* honey, melissopalynology, physicochemical analysis, Portugal.

Introduction

Honey is one of the oldest sweetening agents and is defined as the natural substance produced by *Apis mellifera* bees from the plant nectar, from secretions of living parts of the plants, or from excretions of plant-sucking insects on the living part of plants. Honeybees collect, transform and combine this with specific substances of their own, and then store it and leave it in the honeycomb to ripen and mature (*Codex Alimentarius,* 2001).

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 (Anklam, 1998). Differences in their composition, also mean differences in the organoleptic and nutritional properties of these honeys (Přidal & Vorlová, 2002). The beneficial characteristics of honey are its high nutritional value and the fast absorption of its carbohydrates upon consumption. The importance of understanding the composition of honey from a human health point of view is also valuable. Furthermore, honey was found to be a suitable alternative for healing wounds and burns, and a product that can be used in oral health treatments (Molan, 2001; Lusby *et al*., 2002; Gallardo-Chacon *et al*., 2008; Layflurrie, 2008; Won *et al*., 2009). It also has a potential role in cancer care and shows antimicrobial properties (Bardy *et al*., 2008).

The major consumers and importers of honey are the industrialised countries. A steady rise in consumption in recent years can be attributed to the general increase in living standards and a higher interest in natural and beneficial health products (Arvanitoyannis & Krystallis, 2006). Furthermore, honey is a highly valuable ingredient in sauces, dressings, condiments, beverages and sweet and sour manufactured foods (Rasmussen *et al*., 2008).

Organoleptical properties, physicochemical attributes and pollen spectrum are the main criteria for honey classification. Their measurement is comparatively simple and it provides a good information value. 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 (Abu-Tarboush *et al*., 1993;

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Pérez-Arquillué *et al.*, 1995; Terrab *et al.*, 2002; Lazaridou *et al.*, 2004; Downey *et al.*, 2005; Ouchemoukh *et al.*, 2007).

International legislation is intended to establish the minimum marketing level of the product and the need for consumer protection through correct denominations. Compliance with European Union (EU) legislation (EU, 2002a) may be checked by measuring elemental composition, and requires that the geographical origin of honey be included on packaging. This is essential to protect the consumer from the possible fraudulent mislabelling of inferior honeys. Therefore, the characterisation of honeys is necessary in order to better our response to consumer demands.

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

Figure 1 Map of Portugal showing the distribution of the thirty-one honey samples studied (number of samples): a, Amarante (1); ar, Arouca (2); b, Braga (3); c, Cabeceira de Bastos (4); ca, Castelo de Paiva (5); ce, Celorico de Bastos (6); e, Esposende (7); f, Famalicão (8); fe, Feira (9 and 10); go, Gondomar (11, 12, 13, 14 and 15); g, Guimarães (16); l, Lousada (17); m, Matosinhos (18 and 19); o, Oliveira de Azeméis (20); p, Paços de Ferreira (21, 22 and 23); pa, Paredes de Coura (24, 25 and 26); po, Póboa do Lanhoso (27); t, Terras do Bouro (28); v, Vale de Cambra (29); va, Valongo (30); vi, Vila do Conde (31).

Materials and methods

Honey sampling

Thirty-one (n = 31) honey samples, from *A. mellifera*, were collected by beekeepers from separate apiaries in the *Entre-Douro e Minho* region (Portugal). Figure 1 shows the geographical origin and identification code of the honey samples studied.

Sample floral-type identification

All the samples were subjected to pollen analysis as *per* the method of Louveaux *et al.* (1978). The aim of that analysis was to confirm the floral origin of honey samples. 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 (9:1, Ac_2O, H_2SO_4) 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. Twelve millilitres 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 was carried out with a Leitz Diaplan microscope (Leitz Messtechnik GmbH, Wetzlar, Germany) at 400 × and 1000 × in order to make a 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 from 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 Association of Official Analytical Chemists (AOAC, 1990) and The Harmonised Methods of the European Honey Commission (Bogdanov *et al.*, 1997), reported previously in detail (Pires *et al.*, 2009). The evaluated parameters were: water content (g per 100 g), ash (g per 100 g), electrical conductivity (mS cm^{-1}), hydroxymethylfurfural content (mg kg^{-1}), free acidity (meq kg^{-1}), diastase activity (Gothe degrees), reducing sugars (g per 100 g), apparent sucrose (g per 100 g) and pH. Samples were analysed using the same methods during the same time period to

Table 1 Frequency classes (presence, range and mean ± SD) of the pollen types in the honeys studied

Family	Pollen type	Presence (n=sample size)	Range (%)	Mean ± sd (%)
Myrtaceae	*Eucalyptus*	31	44–79	57.5 ± 9.8
Rosaceae	*Rubus*	25	3–25	10.4 ± 6.1
Ericaceae	*Erica*	21	4–40	15.7 ± 8.7
Fabaceae	*Trifollium*	15	3–14	6.9 ± 2.7
Cistaceae	*Cistus*	10	4–15	8.2 ± 3.1
Boraginaceae	*Echium*	10	6–13	7.9 ± 2.2
Pinaceae	*Pinus*	8	4–10	6.4 ± 2.1
Rosaceae	*Prunus*	8	3–25	11.6 ± 8.1
Fabaceae	*Cytisus*	7	3–11	6 ± 3.1
Fabaceae	*Medicago*	5	4–10	7 ± 2.8
Fabaceae	*Genista*	4	3–8	5.5 ± 2.9
Fabaceae	*Lotus*	4	4–21	10.7 ± 7.9
Fagaceae	*Quercus*	4	5–10	7.5 ± 2.4
Fabaceae	*Acacia*	2	11	11 ± 0
Fagaceae	*Castanea*	2	8	8 ± 0
Fabaceae	*Chamaespartium*	2	3–8	7.5 ± 3.5
Rutaceae	*Citrus*	2	5–10	7.5 ± 3.5
Rosaceae	*Malus*	2	8–10	9 ± 1.4
Malvaceae	*Tilia*	2	8–13	10.5 ± 3.5
Brassicaceae	*Brassica*	1	25	25 ± 0
Brassicaceae	*Diplotaxis*	1	8	8 ± 0
Labiatae	*Lavandula*	1	6	6 ± 0
Rosaceae	*Pyrus*	1	5	5 ± 0

ensure uniform conditions and comparability. Three replicate analyses were made from each sample to obtain the reported data.

Results and discussion

Pollen analysis

Table 1 shows the occurrence frequency of the twenty-three pollen types identified from the thirty-one samples studied. Pollen grains of *Eucalyptus* sp. were found in all honey samples (100%) and ranged from 44% to 79%. This confirms the nectariferous and polliniferous potential of this abundantly blooming, exotic genus cultivated in the reforestation areas of this region. Similar data had already been obtained in previous studies in the *Douro* region (Aira *et al.*, 1998). Eucalyptus is a very important plant from an apicultural point of view (Porter, 1978; D'Arcy *et al.*, 1997; Daners & Tellería, 1998). Its honey production potential varies according to species and location; thus, some authors indicate values per hectare close to 100 kg ha^{-1} and productions per bee colony oscillating between 20 and 100 kg year^{-1} (Goodman, 1973).

The Fabaceae and Rosaceae families provided the greatest number of pollen types with seven (*Acacia, Cytisus, Chamaespartium, Genista, Lotus, Medicago* and *Trifolium*) and four (*Malus, Prunus, Pyrus* and *Rubus*) pollen types each, respectively. *Rubus, Erica* and *Trifolium* are present in twenty-five, twenty-one and fifteen samples, respectively, corresponding to 80.6%, 67.7% and 43.4% of the total samples analysed in percentages. *Rubus*, *Erica* and *Trifolium* are very common plants in the area of study, and their presence in the honey in large amounts is to be expected. Finally, the *Cistus* and *Echium* pollen types are present in ten samples each, corresponding to a 32.3% of the total honeys.

Bees forage different plants; thus, honey is always a mixture of several sources. However, in food control, pollen analysis is very efficient for the differentiation of honeys produced in distinctly different geographical and climatic areas (Anklam, 1998). For example, *Eucalyptus* honeys from West Andalucía and Extremadura (Spain) always contain *Helianthus* pollen (Serra, 1989). Of more than 700 Eucalyptus species in the world, only two (*Eucalyptus globulus* and *Eucalyptus camaldulensis*) are used for afforestation in the Iberian Peninsula in the production of wood pulp. *Eucalyptus globulus* is abundant all along the Cantabrian Coast, the Atlantic Coast and throughout the Northwest Iberian Peninsula, while *E. camaldulensis* is more abundant in the South and Southwest (Seijo *et al.*, 2003).

Usually, honeys are classified as monofloral when the pollen frequency of one plant is over 45%. However, in some cases, the botanical classification is carried out in a different manner when the pollen grains are 'under- or over-represented' in relation to the nectar their flowers yield. This criterion was used to characterise the honeys analysed. In the case of *Eucalyptus* and *Citrus*, which are over- and under-represented taxa, we used values of 70% and 10%, respectively. Five samples (numbers 10, 11, 12, 14 and 22) are listed as *Eucalyptus* honey, one sample (number 28) as *Citrus* honey and twenty-five samples as multifloral honeys (Fig. 2). Citrus honey is produced by putting beehives

Figure 2 Floral declaration of the samples studied. Monofloral *Eucalyptus* honey (*Eucalyptus* pollen presence ≥70%) samples: 10, 11, 12, 14 and 22. Monofloral *Citrus* honey (*Citrus* pollen presence ≥10%) sample 28. Multifloral honeys: the rest of honeys, with a total of twenty-five samples.

in the orange groves during bloom, which also pollinates seeded citrus varieties. Normally, oranges are a winter product; however, Terras de Bouro has a special climate. It is relatively dry all year long and the moisture content is especially low during the winter months. Consequently, oranges are on the trees from March to August. Orange producers are quite interested in obtaining a special domination for the citrus produced in this area, which is currently called 'Laranja de Amares'. This orange is especially sweet and greatly appreciated by consumers.

The economic importance of pollination is often credited entirely to honeybees, and is frequently used to justify public subsidising of honey beekeeping. The economic importance of crop pollination by honey bees is well known, for both the crop and the honey yields. A collaborative agri-environment scheme between beekeeper and orange producers will provide an opportunity to enhance pollinator populations in farmland. Furthermore, orange blossom honey is cherished by consumers. Government policies in Europe now place emphasis on combining the goals of agriculture and conservation, and subsidies are currently available to remove land from arable production (Goulson, 2003).

Physicochemical parameters

Table 2 summarises the results obtained (mean, range and standard deviation, SD) from physicochemical analysis of the honey samples.

Table 2 Physicochemical parameters (moisture, ash, pH, free acidity, electrical conductivity, hydroxymethylfurfural content, apparent sucrose, reducing sugars, and diastase activity) of the analysed honey samples

Sample	Moisture (% w/w)	Electrical conductivity (mS cm^{-1})	Ash (% w/w)	HMF (mg kg^{-1})	Diastase activity (Gothe Scale)	pH (pH units)	Free acidity (meq kg^{-1})	Reducing sugars (%)	Apparent sucrose (%)
1	17.6	0.52	0.20	28.6	20	3.78	28.0	76.92	3.53
2	16.1	0.61	0.25	5.2	10	3.54	26.8	71.43	4.11
3	17.8	0.72	0.31	8.1	15	4.04	31.0	68.49	4.27
4	17.5	0.64	0.26	1.5	15	3.91	21.6	71.43	4.33
5	16.0	0.48	0.17	5.1	15	3.96	17.1	72.99	3.73
6	18.1	0.61	0.25	3.2	15	3.54	29.9	72.99	4.30
7	17.6	0.73	0.31	7.6	25	3.72	29.6	76.92	3.53
8	16.8	0.58	0.23	8.0	12	3.85	21.4	71.43	3.57
9	16.8	0.52	0.20	8.3	15	3.45	22.7	80.00	4.50
10	16.5	0.45	0.16	9.5	12	3.29	23.6	74.07	3.85
11	15.8	0.39	0.12	8.9	12	4.03	23.3	72.46	4.24
12	16.8	0.57	0.22	7.4	18	3.46	29.3	76.92	3.53
13	16.8	0.51	0.19	4.5	10	3.73	21.3	72.99	3.73
14	16.5	0.43	0.15	6.9	15	3.60	19.6	75.76	3.42
15	16.5	0.55	0.22	2.5	13	3.89	19.2	73.53	4.36
16	17.2	0.52	0.20	2.4	14	3.84	19.9	64.52	4.22
17	17.0	0.61	0.25	5.3	10	3.49	31.0	74.07	3.85
18	16.8	0.60	0.24	8.0	20	3.63	24.8	74.07	3.85
19	18.1	0.76	0.33	12.9	25	3.44	48.9	72.46	4.24
20	16.8	0.49	0.18	7.1	15	3.52	28.9	68.03	3.23
21	17.7	0.46	0.17	1.6	15	3.81	18.9	74.07	3.85
22	17.1	0.52	0.20	9.2	20	3.77	27.3	71.43	3.99
23	17.0	0.45	0.16	3.6	15	3.85	26.4	72.46	3.13
24	16.8	0.45	0.16	3.5	20	3.75	28.0	72.46	4.24
25	17.0	0.51	0.19	6.1	20	3.73	21.1	74.07	3.85
26	17.2	0.52	0.20	7.8	15	3.76	21.0	76.33	4.09
27	17.5	0.54	0.21	5.0	17	3.82	20.3	69.44	4.40
28	17.1	0.49	0.18	2.2	12	4.06	24.5	73.53	4.36
29	16.8	0.46	0.17	5.0	15	3.70	27.1	66.67	2.63
30	18.3	0.61	0.25	3.3	12	3.57	32.9	75.19	3.96
31	17.7	0.48	0.17	1.7	15	3.62	28.2	71.43	3.57
Mean	17.1	0.54	0.21	6.5	15	3.71	25.6	72.86	3.89
V_{min}	15.8	0.39	0.12	1.5	10	3.29	17.1	64.52	2.63
V_{max}	18.3	0.76	0.33	28.6	25	4.06	48.9	80.00	4.50
SD	0.61	0.09	0.05	4.97	3.90	0.19	6.08	3.20	0.43

V_{min}, minimum value; V_{max}, maximum value.

The moisture content (%) varied from 15.8 to 18.3 (mean value ± SD = 17.1 ± 0.61%) indicating optimum harvesting and a good degree of maturity. The mean value obtained was similar when compared to honeys from 'Serra da Lousã', Portugal (17.8%; Andrade *et al.*, 1999), Italian honeys from the Marche región (17.4%; Conti *et al.*, 2007), Italian honeys (17.3%; Zappalà *et al.*, 2005) or North Argentine honeys (17.4%; Baroni *et al.*, 2009). However, the mean result (16.2% w/w) was higher than that obtained by Mendes *et al.* (1998) when investigating honeys produced in different regions of Portugal. In the *Codex Alimentarius* (2001) Standard and EU (2002a) Council directives, the maximum water content value of pure floral honey is less 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, water content is also a value 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 (Acquarone *et al.*, 2007). The small variation observed in the water contents of these samples may be due to the similar beehive handling practices applied by Portuguese beekeepers.

The designation 'fresh', 'raw' or 'virgin' honey has been proposed by European legislation to indicate the virginal (pure and natural) nature of honey and to its wholeness (nothing added, removed or altered) (EU, 2002b). It is identified by physical and chemical requirements that are more restrictive than those under Community law (maximum humidity of 18% and maximum hydroxymethylfurfural (HMF) content of 25 mg kg^{-1}). Of the total honey studied, 87.1% can be labelled with the 'virgin' label of distinction. Ash content fraction is a useful parameter in determining botanical origin of honey and differentiating between nectar honey and honeydew (White, 1978); blossom honeys have a lower (≤0.6%) ash content than honeydew honeys (≤1.2%). Blossom, nectar or floral honey is honey which is produced from the nectar of plants, whereas honeydew honey is obtained mainly from the excretions of plant sucking insects (*Hemiptera*) on the living part of plants or secretions of living parts of plants. Ash values were below 0.6%, as expected for nectar honeys (*Codex Alimentarius*, 2001; EU, 2002a). The honeys considered in this study had ash contents ranging from 0.12 to 0.33.

Electrical conductivity varies with botanical origin (Terrab *et al.*, 2003); floral honeys should have conductivity values below 0.8 mS cm^{-1}; while honeydew should have values over 0.8 mS cm^{-1}. All samples had conductivity measurements below 0.8 mS cm^{-1}; this suggests once again that honeys collected for this study were of floral origin. Conductivity values ranged from 0.39 to 0.76 mS cm^{-1} (mean value ± SD = 0.54 ± 0.09 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 mean conductivity value obtained for the thirty-one samples in this study is similar to the previously published values for Portuguese honeys of 0.52 mS cm^{-1} (Andrade *et al.*, 1999).

Diastase activity and HMF are widely recognised as parameters 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}. It is well known that heating of honey results in the formation of HMF, which is produced during acid-catalysed dehydration of hexoses, e.g. fructose and glucose (Belitz & Grosch, 1999).The HMF content of the honeys analysed ranged from 1.5 to 28.6 mg kg^{-1} (mean value ± SD = 6.5 ± 4.97 mg kg^{-1}). The HMF content is indicative of honey freshness (Terrab *et al.*, 2002), 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 15 (Gothe degrees) (average) with a range of 10–25 and a SD of 3.90 (Gothe degrees). Values obtained for HMF and diastase activity 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 (Anklam, 1998). Furthermore, diastase activity has been related to the origin of the samples (Persano-Oddo *et al.*, 1990; Persano-Oddo & Piro 2004). No sample exceeded the limits established for these variables. Since the honey samples were collected during the same period, differences in HMF could be attributed to the variation in climatic conditions of the area.

Honey pH is of great importance during the extraction and storage of honey as it influences the texture, stability and shelf life of honey (Terrab *et al.*, 2004). Published reports indicate that pH should be between 3.2 and 4.5 (Bogdanov *et al.*, 1999). The values of pH in honey help determine its origin: flower or forest; latter show higher values. Values recorded for this parameter in the current study ranged from 3.29 to 4.06, with an average of 3.71. 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. The pH values recorded were less than those obtained by Andrade *et al.* (1999) for floral honeys collected in Portugal, for which a mean value of 4.1 was obtained.

The free acidity of honey samples is 25.6 meq kg^{-1} (average) with a range of 17.1–48.9 and a SD of 6.08 meq kg^{-1}. Free acidity may be explained by taking into account the presence of organic acids, which are proportional to the corresponding lactones, or internal esters, and some inorganic ions such as phosphate or sulphate (Finola *et al*., 2007). Variation in free acidity among different honeys can be attributed to floral origin or to variation because of the harvest season (Pérez-Arquillué *et al*., 1994). 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). This indicates the absence of unwanted fermentations.

Honey is mainly composed of the monosaccharides glucose and fructose. The reducing sugars (%) content of the honeys analysed ranged from 64.52% to 80.00% (mean value ± SD = 72.86 ± 3.20) and the mean percentages of apparent sucrose (%) is 3.89% with a range of 2.63–4.50 and a SD of 0.43 (according to European Directives, sucrose content must be under 5%). These two parameters confirm that the honey samples studied were floral honeys and had a good maturation grade.

Relationship between electrical conductivity and ash parameters

Ash and electrical conductivity values depend on the mineral content of the honey: ash gives a direct measure of inorganic residue after carbonisation, while electric conductivity measures all ionisable organic and inorganic substances. The relationship between these two parameters has been demonstrated by several researchers for Italian honeys ($y = 1.74x + 0.14$; Piazza *et al*., 1991), Irish honeys ($y = 0.771 + 0.1084$; Downey *et al*., 2005), Slovenian honeys ($y = 2.03x + 0.14$; Kropf *et al*., 2008) and Basque honeys ($y = 1.205x + 0.092$; Sancho *et al*., 1991). The first model has been proposed for use around all of Europe by the International Honey Commission (IHC; Bogdanov *et al*., 1997).

This model should replace an older and more time-consuming method used to determine total ash mass fraction by ashing. Confirmation of this relationship, in the honeys analysed, is revealed in Fig. 3. The present study found a linear correlation ($R = 0.995$) between the specific conductivity of honeys and their ash content. The final regression model obtained ($y = 0.551x$–0.089) is shown graphically in Fig. 3.

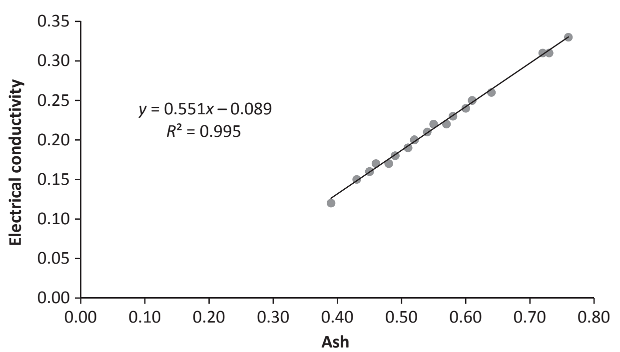

Figure 3 Linear relationship between ash content (%) and electrical conductivity (mS cm^{-1}) in honey samples from *Entre-Douro e Minho* region.

As an illustration, if an *Entre-Douro e Minho* honey sample from the present study has an electrical conductivity of 0.46 mS cm^{-1}, the ash mash fraction is 0.16 g per 100 g as calculated by the obtained model, 0.18 g per 100 g using the model proposed by the IHC, 0.29 g per 100 g using the model proposed by Sancho *et al*. (1991), 0.46 g per 100 g using the model proposed by Downey *et al*. (2005); or 0.16 g per 100 g using the model proposed by Kropf *et al*. (2008). Observed disparity is an outcome of a different type and different geographical origin of honey used in the research. However, both the IHC and the Slovenian models are directly comparable to the model obtained in our study.

Conclusion

In this work, the principal physicochemical parameters have been determined for honeys from the *Entre-Douro e Minho* region in Portugal. All of the values obtained for the physicochemical parameters analysed in this work fell within the maximum limits defined under current Standard Codex and European legislation. Furthermore, more than 87.1% of the samples should be labelled as 'Virgin Honey' in accordance with EU rules The regression model between the ash fraction and electrical conductivity was $y = 0.551x - 0.089$. The melissopalynological method confirmed the identity of the honey sources. Five honey samples can be classified as monofloral *Eucalyptus*, one as monofloral *Citrus* and the rest, twenty-five honey samples, as multifloral. The latter showed a mean value of 57.5% for *Eucalyptus* pollen. An overall consideration of the samples analysed shows that the most important nectariferous taxa for the area studied are *Rubus, Erica* and *Trifolium* present in 80.6%, 67.7% and 43.4%, respectively, of the total analysed samples. Although this work was a preliminary and limited investigation into the characterisation of honeys produced in the *Entre-Douro e Minho* region, in order to confirm our preliminary results, a larger sample data set is also in preparation. From the economical standpoint, the assessment of floral origin and physicochemical properties may increase the commercial value of these honeys.

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