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Characterization of artisanal honey produced on the Northwest of Portugal by melissopalynological and physico-chemical data

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ABSTRACT

Honey has always been regarded as a food which is advantageous for one's health and as a product which has healing qualities. For this reason, is necessary to protect consumers from the fraudulent mislabeling of inferior honeys. The purpose of this study was to investigate some properties of artisanal honey samples ($n = 45$) collected from the Northwest of Portugal by using different honey analysis tests such as moisture, ash, pH, free acidity, electrical conductivity, hydroxymethylfurfural (HMF), apparent sucrose, reducing sugars and diastase activity. 77.8% of the total exceeded the quality parameters and should be labeled as "virgin" (humidity $\leqslant$18% and HMF $\leqslant$ 25 mg/kg). The present study found a linear correlation ($y = 0.551x - 0.089$; $R = 0.995$) between the electrical conductivity of honeys and their ash content. All of the samples showed an *Erica sp*. pollen percentage $\geqslant$15%, and 42% of the total were monofloral *Erica sp*. In respect to coliforms and *Salmonella*'s presence, all the honey's samples shown to be negative. The existence of sulphite-reducing *Clostridia* was low, and well below the established limit by MERCOSUR. Yeasts, moulds and aerobic mesophiles were detected in low amounts.

1. Introduction

Honey was the first and most reliable sweetener used by human beings. As a source of energy, the beneficial characteristics of honey are its high nutritional value and the fast absorption of its carbohydrates upon consumption (Viuda-Martos et al., 2008). Moreover, the importance of honey in various areas of daily life has been appreciated for centuries and across civilizations. The fact that Hippocrates, the father of medicine, emphasizes the nutritional and pharmaceutical value of honey is not accidental. Many researchers have found honey to be a suitable alternative for healing wounds and burns, and for oral health (Lay-Flurrie, 2008; Molan, 2001a, 2001b). Others have determined its potential role in cancer care as well as its antimicrobial properties (Bardy et al., 2008; Estevinho et al., 2008).

According to the *Codex Alimentarius (2001)*, honey is defined as the natural substance produced by *Apis mellifera* bees from plant nectar, from secretions of living parts of plants, or from 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 honeycomb to ripen and mature.

As a natural, unprocessed and easily digested food, honey can be regarded as an important part of our diet. For these reasons, honey still retains this 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 (Arvanitoyannis and Krystallis, 2006). Since European honey production is insufficient, it is imported in increasing amounts from international markets, mainly Argentina and China. In Europe, imported honey is more economical than the locally produced honey, and is therefore prone to mislabeling for economic reasons. The major concern of honey quality control groups is to ensure that honey is authentic in respect to the legislative requirements. *Codex Alimentarius (2001)* and EU (2002a) legislation are intended to establish the minimum marketing level of the product and the need for consumer protection through correct denominations.

The identification and quantification of pollen grains in honey sediment (melisopallynological) is still the most important method for determining the botanical origin of honey (Anklam, 1998). The development of new alternative methods for determining the geographical origin of honey, such as studies with isotope ratios other than carbon (White, 2000), or electronic tongue (Dias et al., 2008) is still in the beginning stages. Commonly, monofloral honeys are made up of nectar belonging to a single plant in an extent of at least 45%. 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

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producers than honeys from mixed botanical sources and can thus be considered as premium products.

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 (Bianchi et al., 2005). The physico-chemical parameters of natural honeys, such as moisture, sucrose and hydroxymethylfurfural (HMF) contents, acidity and specific conductivity, are strictly defined and constitute the quality indicators which characterize individual honey varieties. Their measuring is comparatively simple and they provide a good information value (Downey et al., 2005; Al-Khalifa and Al-Arify, 1999; Naab et al., 2008; Perez-Arquillué et al., 1995).

The present study aims to characterize artisanal honeys harvested in Northwest Portugal with respect to: (i) floral nectar origin, (ii) physico-chemical parameters (moisture, ash, pH, free acidity, electrical conductivity, HMF content, apparent sucrose, reducing sugars and diastase activity) and (iii) microbial safety (aerobic mesophiles, moulds and yeasts, fecal coliforms, sulphite-reducing Clostridia and *Salmonella*).

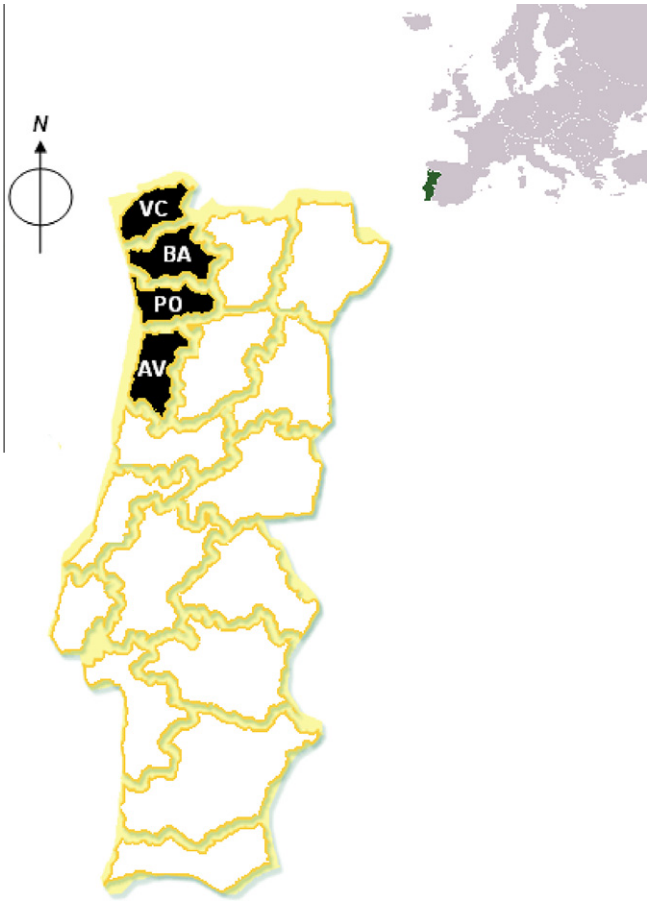

Fig. 1. Map of Portugal showing honey sample regions. AV (Aveiro, samples 1–6), BA (Braga, samples 7–23), PO (Porto, samples 24–33) and VC (Viana do Castelo, samples 34–45).

2. Materials and methods

2.1. Honey sampling

Forty-five ($n = 45$) artisanal honey samples, from *A. mellifera iberica*, were collected by beekeepers from separate hives. They were obtained by centrifugation and stored at 10 °C until analysis. The honeys were harvested from different districts in Northwest Portugal: Aveiro, six samples ($n = 1–6$); Braga, 17 samples ($n = 7–23$); Porto, 10 samples ($n = 24–33$) and Viana do Castelo, 12 samples ($n = 34–45$). Fig. 1 shows the honey sampling regions. A single 100 g jar of honey was homogenized using a Turrax mixer (11,000 rpm) over five 20-s periods separated by 10 min to limit sample heating. The sample was placed onto a roller mixer at 35 rpm for 16 h until the honey was homogeneous.

2.2. Sample floral-type identification

The botanical origin of the samples of honey was based on the pollen spectrum proposed by (Louveaux et al., 1978). Briefly, pollen analyses are based on the extraction of pollen grains from 10 g of 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. 12 mL of 7% KOH is added, stirred thoroughly, centrifuged, and decanted.

Table 1
Frequency classes, presence, range and media of the pollen types in the honeys.

Family	Pollen type	PP[a]	SP[b]	IMP[c]	MP[d]	Presence	Range (%)	Mean ± SD (%)
Fabaceae	Acacia	–	1	4	–	5	5–17	9.4 ± 5.0
	Cytisus	–	5	4	–	9	6–20	14.7 ± 5.4
	Chamaespartium	–	–	6	–	6	3–15	6.3 ± 4.5
	Genista	–	2	4	–	6	3–16	9.7 ± 5.8
	Lotus	–	–	3	–	3	5–11	7.7 ± 3.1
	Medicago	–		8	–	8	4–8	6.0 ± 1.3
	Trifolium	–	4	23	–	27	4–23	9.5 ± 5.4
	Vicia	–		5	–	5	4–8	6.2 ± 1.6
Rosaceae	Prunus	–	1	11	–	12	3–21	8.3 ± 5.2
	Pyrus	–	–	1	–	1	6	6.0 ± 6.0
	Rubus	–	4	33	–	37	3–20	9.4 ± 4.6
Asteraceae	Carduus	–	1	2	–	3	7–22	12.3 ± 8.4
Brassicaceae	Brassica	–	–	1	–	1	12	10.0 ± 0
Rutaceae	Citrus	–	–	1	–	1	6	6.0 ± 0
Cistaceae	Cistus	–	1	19	–	20	4–20	8.0 ± 4.3
Boraginaceae	Echium	–	2	15	–	17	4–20	8.1 ± 4.6
Ericaceae	Erica	20	25	–	–	45	30–71	46.6 ± 12
Myrtaceae	Eucalyptus	1	26	9		36	6–48	21.3 ± 9.7
Labiatae	Lavandula	–	–	3	–	3	7–8	7.3 ± 0.6
Pinaceae	Pinus	–	–	10	–	10	3–10	5.9 ± 2.2
Fagaceae	Quercus	–	3	8	–	11	5–21	10.4 ± 5.8

[a] PP, predominant pollen (>45%).
[b] SP, secondary pollen (16–45%).
[c] IMP, important minor pollen (3–15%).
[d] MM, minor pollen (1–3%).

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 an optical microscope at 400× and 1000× in order to make a sound identification of the pollen types. A minimum of 1200 pollen grains was counted per sample. In order to recognize 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. The following terms were used for frequency classes: predominant pollen (PP, more than 45% of pollen grains counted), secondary pollen (SP, 16–45%), important minor pollen (IMP, 3–15%) and minor pollen (MP, 1–3%).

2.3. Physico-chemical analysis

Physico-chemical 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). Samples were analyzed during the same time period to ensure uniform conditions and comparability.

The determination of moisture (*M*) (AOAC Official Method 969.38) was ascertained by refractometry, using an Abbe refractometer (Digital refractometer Atago, Germany). All measurements were performed at 20 °C, after waiting for 6 min for equilibration, and obtaining the corresponding% moisture (g/100 g honey) from the refractive index of the honey sample by reference to a standard table.

Electrical conductivity (EC) of a honey solution at 20% (w/v) (dry matter basis) in CO_2-free deionized distilled water was measured at 20 °C in a Crison 522 conductimeter. Results were expressed as milliSiemens per centimeter (mS/cm).

The method used in the experiments to determine the mineral content and other inorganic matter in honey (AOAC Official Method 920.181) consisted of the desiccation of an amount of 5 g, for each honey sample, in a platinum dish. To do so, they were kept in the thermostat at 80 °C for 4 h, after which the samples underwent calcination at 550 °C, in an 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/100 g honey), was calculated from the following equation:

$$\text{Ash}(\%) = [(m_1 - m_2)/m_0] \times 100 \tag{1}$$

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.

HMF was determined by the standard method (AOAC Official Method 980.23). 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% (m/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, USA). HMF contents, expressed as mg/kg, were calculated from the following equation:

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

where A_{284}, $A_{236}m_1$ are the absorbance readings and *F* = 149,7 was calculated with following equation:

$$F = (126 \times 1000 \times 1000)/(16830 \times 10 \times 5) \tag{3}$$

where 126 is the molecular weight of HMF and 16,830 the molar absorptivity of HMF at 284 nm.

Diastase activity (DA) (AOAC Official Method 958.09) 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 (Norwalk, USA). 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.

Five grams of honey samples were diluted with 20 mL of distilled water and mixed thoroughly (AOAC Official Method 962.19). The pH values for these samples were measured using Digital pH Meter (pH-526 WTW Germany).

Free acidity (FA) (AOAC Official Method 962.19) 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 tritated to pH 8.5 by adding 0.05 N NaOH solution.

Reducing sugars (RS) were determined by reducing Soxhleťs modification of Fehlinǵs 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 (AS) content.

Table 2
Pollen spectrum of honeys studied. Data presented are about pollen percentage per sample.

Family	Pollen type	Honey sample 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*	–	6	–	–	–	–	–	–	–	–	–	–	12	–	–	–	–	–	–	–	–	–	–
	Cytisus	–	–	–	–	–	–	6	20	20	17	–	13	–	–	–	–	–	–	–	–	–	–	–
	Chamaespartium	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4
	Genista	–	–	–	–	5	–	–	–	–	–	–	–	–	–	6	–	–	–	–	–	–	–	–
	Lotus	–	–	–	–	–	7	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–
	Medicago	–	6	–	–	–	–	–	–	–	–	5	–	–	5	–	–	4	–	–	–	8	–	7
	Trifolium	7	22	7	8	5	4	6	13	–	–	16	4	6	12	6	15	11	–	–	–	23	17	4
	Vicia	–	–	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	7	–	–	–
Rosaceae	*Prunus*	–	–	–	–	–	–	–	–	7	–	–	4	–	5	12	–	–	11	4	–	–	–	–
	Pyrus	–	–	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–
	Rubus	7	6	7	–	20	13	6	7	7	8	11	4	12	5	6	–	–	16	8	4	15	17	15
Asteraceae	*Carduus*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8	–	–
Brassicaceae	*Brassica*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	12	–	–	–
Rutaceae	*Citrus*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cistaceae	*Cistus*	12	6	–	4	–	–	–	–	13	–	–	–	6	5	–	–	4	6	4	–	–	–	–
Boraginaceae	*Echium*	–	–	7	4	–	–	6	13	20	8	16	4	–	–	12	–	4	6	4	4	8	–	–
Ericaceae	*Erica*	56	32	66	71	30	37	70	40	33	42	47	46	46	37	34	46	63	50	56	50	38	33	35
Myrtaceae	*Eucalyptus*	18	22	13	13	40	33	–	7	–	17	–	25	18	31	24	23	14	11	24	12	–	33	35
Labiatae	*Lavandula*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pinaceae	*Pinus*	–	–	–	–	–	3	6	–	–	8	–	–	–	–	–	8	–	–	–	4	–	–	–
Fagaceae	*Quercus*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8	–	–	–	7	–	–	–

Family	Pollen type	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Fabaceae	*Acacia*	7	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	17
	Cytisus	7	–	–	–	–	–	–	–	–	13	–	16	–	20	–	–	–	–	–	–	–	–
	Chamaespartium	–	–	–	3	–	–	–	–	–	15	–	6	7	–	–	–	–	–	–	–	–	–
	Genista	–	–	–	3	–	–	–	–	–	–	–	16	–	–	–	–	–	12	–	–	16	–
	Lotus	–	11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Medicago	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6	7	–	–	–
	Trifolium	–	–	–	–	6	–	7	7	–	6	14	–	–	13	5	–	–	6	7	–	–	–
	Vicia	–	–	4	–	–	–	–	–	8	–	–	–	–	–	–	–	–	–	7	–	5	–
Rosaceae	*Prunus*	21	–	–	3	–	5	–	–	–	–	–	–	–	–	–	–	5	11	–	–	–	11
	Pyrus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6	–	–	–	–	–	–
	Rubus	7	11	11	3	6	20	7	13	8	6	14	–	–	–	5	6	11	6	–	15	–	5
Asteraceae	*Carduus*	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	22	–	–	–
Brassicaceae	*Brassica*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Rutaceae	*Citrus*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6	–	–	–	–	–	–
Cistaceae	*Cistus*	–	5	11	–	–	10	7	–	8	–	–	–	7	20	5	–	5	–	7	15	–	–
Boraginaceae	*Echium*	–	–	–	–	–	–	–	7	–	–	–	–	–	–	9	–	–	–	–	–	–	6
Ericaceae	*Erica*	30	37	44	40	65	40	65	33	45	33	44	56	44	40	67	70	42	59	43	50	53	39
Myrtaceae	*Eucalyptus*	21	26	26	48	17	25	7	33	23	27	14	–	21	7	–	6	16	–	–	–	16	22
Labiatae	*Lavandula*	–	–	–	–	–	–	7	7	8	–	–	–	–	–	–	–	–	–	–	–	–	–
Pinaceae	*Pinus*	–	5	4	–	–	–	–	–	–	–	–	–	–	–	–	6	–	–	–	10	5	–
Fagaceae	*Quercus*	–	–	–	–	6	–	–	–	–	–	14	6	21	–	9	–	21	–	7	19	5	–

2.4. Microbiological analysis

Ten grams of each organic honey sample were weighed aseptically and homogenized with 90 mL of sterile peptone water (10^{-1} dilution) in a stomacher bag. Subsequent decimal dilutions were made into the same solvent.

Aerobic mesophilic bacteria were counted onto standard plate count agar (PCA) and incubated at 30 °C for 48 h. Mould and yeast counts followed the protocol of ISO 21527-2:2008. Microbial counts were expressed as colony-forming units per gram of honey (cfu/g). For sulphite-reducing Clostridia counting, aliquots of 10, 5, 1 and 0.1 mL of the initial suspension were added to an empty tube, thermally treated at 80 °C for 5 min and covered with SPS (sulphite–polymixin–sulfadiazine) agar media, tubes were incubated at 37 °C for 5 days. Then fecal coliforms and *Salmonella* detection were analyzed. Fecal coliforms were enumerated by the Most Probable Number technique defined in the protocol. *Salmonella* detection followed the protocol of ISO 6579:2002(E). All microbial tests were performed in triplicate (Gomes et al., 2010).

3. Results and discussion

3.1. Pollinic analysis

The results of the honey pollen analysis are shown in Tables 1 and 2. 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).

Table 1 shows the occurrence frequency of the 21 pollen types identified from the 45 studied samples. The *Fabaceae* and *Rosaceae* families provided the greatest number of pollen types with 8 (*Acacia, Cytisus, Chamaespartium, Genista, Lotus, Medicago, Trifolium*

Table 3
Physico-chemical parameters of honey samples analyzed: moisture (M), electrical conductivity (EC), ash, hydroxymethylfurfural (HMF), diastase activity (DA), pH, free acidity (FA), reducing sugars (RS) and apparent sucrose (AS).

Sample	M(%)	EC(mS/cm)	Ash(%)	HMF(mg/kg)	DA	pH	FA(meq/kg)	RS(%)	AS(%)
1[a]	18.1	0.79	0.35	16.3	25	3.7	39.2	74.1	3.8
2[b]	17.0	0.63	0.26	6.7	12	3.6	27.7	76.9	3.5
3[a]	17.5	0.79	0.35	22.5	20	3.7	39.3	73.5	3.2
4[a]	17.5	0.69	0.29	7.1	15	3.9	25.7	71.4	4.1
5[b]	18.1	0.52	0.20	10.3	23	3.5	21.0	70.4	2.9
6[b]	17.5	0.58	0.23	8.3	12	3.9	27.7	69.0	4.3
7[a]	18.1	0.70	0.30	2.8	15	3.9	33.4	71.4	3.0
8[b]	16.9	0.54	0.21	2.5	12	3.8	28.6	76.9	3.5
9[b]	17.0	0.69	0.29	9.3	12	3.8	34.3	72.5	4.2
10[b]	17.3	0.70	0.30	9.5	15	3.8	34.3	74.1	3.8
11[a]	17.5	0.75	0.32	0.9	12	4.1	31.7	73.0	3.7
12[a]	17.5	0.67	0.28	4.4	20	3.8	31.9	74.1	3.8
13[a]	17.2	0.52	0.20	1.7	14	3.9	21.9	75.8	3.4
14[b]	17.2	0.61	0.25	0.5	11	4.1	22.1	74.1	4.4
15[b]	17.0	0.48	0.17	0.4	30	3.9	17.2	73.0	3.6
16[a]	17.2	0.64	0.26	1.9	12	4.1	25.3	76.9	3.5
17[a]	18.1	0.67	0.28	1.8	20	4.0	30.2	73.5	3.8
18[a]	17.2	0.67	0.28	8.4	20	4.0	27.1	68.5	4.3
19[a]	17.1	0.61	0.25	1.4	25	3.9	27.4	69.9	4.5
20[a]	17.5	0.94	0.43	13.6	20	4.2	34.0	70.4	4.0
21[b]	18.6	0.55	0.22	13.1	12	3.5	35.6	71.4	3.0
22[b]	16.9	0.69	0.29	4.7	15	3.9	26.2	74.1	3.8
23[b]	17.7	0.63	0.26	9.3	13	3.8	28.6	74.1	3.8
24[b]	17.2	0.49	0.18	5.5	12	3.6	22.0	71.4	3.0
25[b]	18.1	0.67	0.28	7.3	23	3.7	34.3	74.6	2.7
26[b]	18.3	0.67	0.28	5.8	15	3.6	36.0	71.4	3.0
27[b]	18.3	0.61	0.25	3.3	12	3.6	32.9	75.2	4.0
28[a]	17.5	0.73	0.31	2.3	12	3.9	31.1	73.0	4.3
29[b]	18.0	0.64	0.26	7.6	15	3.7	30.0	73.0	3.7
30[a]	17.8	0.75	0.32	2.5	13	4.0	29.8	74.1	4.4
31[b]	16.8	0.58	0.23	11.4	20	3.6	28.7	73.5	3.2
32[a]	18.0	0.61	0.25	22.8	23	3.5	45.2	72.5	4.2
33[b]	17.0	0.46	0.17	5.8	13	3.8	21.7	70.4	4.0
34[b]	17.3	0.63	0.26	21.0	25	3.6	36.4	74.1	3.8
35[a]	17.2	0.70	0.30	9.2	12	3.7	36.9	74.1	3.8
36[b]	18.0	0.88	0.40	7.7	20	3.9	37.2	69.9	3.9
37[b]	18.0	0.67	0.28	2.9	15	3.8	32.9	72.5	3.1
38[a]	17.2	0.73	0.31	1.7	20	4.0	23.5	74.1	3.8
39[a]	17.3	0.73	0.31	0.9	15	3.9	26.7	73.0	3.7
40[b]	18.1	0.69	0.29	8.9	12	3.7	30.3	71.4	3.0
41[a]	18.1	0.76	0.33	6.2	30	4.0	33.2	66.7	3.6
42[b]	17.1	0.76	0.33	0.2	12	4.2	23.6	70.4	4.0
43[a]	18.0	0.64	0.26	2.8	10	3.8	29.8	69.4	3.9
44[a]	17.0	0.70	0.30	5.8	25	3.9	24.2	69.9	3.4
45[b]	16.8	0.67	0.28	8.1	25	3.9	25.1	73.5	4.4
	M(%)	EC(mS cm^{-1})	Ash(%)	HMF(mg kg^{-1})	DA	pH	FA(meq kg^{-1})	RS(%)	AS(%)
mean	17.5	0.66	0.28	6.8	17	3.8	29.8	72.6	3.7
Range±SD	(16.8–18.6) ± 0.5	(0.46–0.94) ± 0.10	(0.17–0.43) ± 0.05	(0.2–22.8) ± 5.7	(10–30) ± 6	(3.5–4.2) ± 0.2	(17.2–45.2) ± 5.8	(66.7–76.9) ± 2.3	(2.7–4.5) ± 0.5
[a]mean	17.6	0.70	0.30	6.5	18	3.9	30.8	72.3	3.8
[a]range±SD	(17.0–18.1) ± 0.4	(0.52–0.94) ± 0.08	(0.20–0.43) ± 0.05	(0.9–22.8) ± 6.8	(10–30) ± 6	(3.5–4.2) ± 0.2	(21.9–45.2) ± 5.9	(66.7–76.9) ± 2.5	(3.0–4.5) ± 0.4
[b]mean	17.5	0.63	0.26	7.1	16	3.8	28.9	72.8	3.6
[b]range±SD	(16.8–18.6) ± 0.6	(0.46–0.88) ± 0.09	(0.17–0.40) ± 0.05	(0.2–21.0) ± 4.6	(11–30) ± 5	(3.5–4.2) ± 0.2	(17.2–37.2) ± 5.7	(69.0–76.9) ± 2.1	(2.7–4.4) ± 0.5

[a] Monofloral *Erica* samples found (*Erica* pollen percentage > 45%).
[b] Multifloral samples found.

and *Vicia*) and 3 (*Prunus, Pyrus* and *Rubus*) pollen types each, respectively. *Rubus* and *Trifolium* are present as IP in 33 and 23 samples, respectively, corresponding to 73% and 51% ot the total analysed samples in percentages, and as SP *Rubus* and *Trifolium* are present in four samples, respectively. A full spectrum analysis of the total honeys with the corresponding pollen percentage per sample is given in Table 2. The Portuguese honeys analyzed have between four (sample 22) and eight (sample 20) pollen types, the mean number being 5.9 with a standard deviation of 0.8.

Erica sp. pollen is present in all honey samples, as PP (in 20 samples) and as SP (in 25 samples, corresponding to a 55% of the total honeys). Next, the *Eucalypthus* pollen type is present as PP in one sample and as SP and IMP in 26 and 9, honeys respectively. Monofloral honeys are made up of nectar belonging to a single plant in an extent of at least 45%. The final results indicated that 42% of all the samples were monofloral *Erica sp.* honeys. 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 characterized by its dark brown color, strong flavor and a slightly salty taste. Consumers in Portugal prefer heather honeys and they are generally more costly than others (Andrade et al., 1999). From the economical standpoint, the assessment of a monofloral origin may increase the commercial value of these honeys (Pires et al., 2009). 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. Currently, in the EU, Portugal has the highest number of honeys bearing the Protected Designation of Origin (PDO) logo, which are produced, processed and prepared in a given geographical area using certified know-how (EU, 2006). The technical requirements of the "Terras Altas do Minho" Portuguese honey, which is recognized as a PDO honey in the EU, express that only 15% of *Erica sp.* pollen is necessary to declare this product to form part of this protected denomination. Furthermore, honey samples with an *Erica sp*. pollen percentage higher than 35% could be labeled with a specific "Mel de Urze" or "Mel de Queirós" denomination. All of the samples analyzed in the present study, have an *Erica sp*. pollen percentage higher than 15% and only eight samples fall below 35%.

3.2. Physico-chemical parameters

Visually, all honey samples showed no sign of fermentation or granulation before initiating the physico-chemical analysis. Table 3 shows the results obtained (mean, range and standard deviation, SD) from physico-chemical analysis of the multifloral and monofloral *Erica* honey samples.

Knowledge of the *M* contents in honey is useful to improve its conservation and storage by preventing the growth of molds such as *Penicillium* and *Mucors* on its surface. If the mold does grow, it then ferments, resulting in a product with an off-taste, high levels of dead yeast, and glycerol, butanediol and ethanol that reduce the quality of this product.

Furthermore, the water content value is also of great importance because it is considered to be a useful parameter for describing moistness and viscosity of honey. The *M* (%) varied from 16.8 to 18.6 (mean value ± standard deviation = 17.5 ± 0.5%). The small variation observed in the water contents of these samples may be due to the similar bee-hive handling practices applied by Portuguese beekeepers. In *Codex Alimentarius (2001)* and EU (2002a) Council directives the maximum *M* content value of pure floral honey is given as 23% for heather honeys and not more than 20% in general. 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 maximum amount of *M* present in honey is the only composition criteria which as a part of the Honey Standard, has to be met for all world trade honeys.

HMF and DA 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 DA, and a maximum HMF content of 40 mg/kg (Codex Alimentarius, 2001; EU, 2002a). The HMF content of the honeys analyzed ranged from 0.2 to 22.8 mg/kg (mean value ± standard deviation = 6.8 ± 5.7 mg/kg). The HMF content is indicative of honey freshness (Terrab et al., 2002), and from this point of view most of the analyzed samples are fresh, and thus, coincide with the information provided by the producers. The DA of honey samples is 17 (Gothe degrees) (average) with a range of 10–30 and a standard deviation of 6 (Gothe degrees). 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 (Anklam, 1998). No sample exceeded the limits established for these variables (Codex Alimentarius, 2001; EU, 2002a).

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 HMF content of 25 mg/kg). 77.8% of the total honey studied can be labeled with the "virgin" label of distinction. The HMF values of all the samples tested are under 25 mg/kg. Only ten of the total of the honeys analyzed have M values higher than the legal requirement, but even these do not deviate greatly from the maximum required since they show a maximum *M* content above 18.6%. Fig. 2 shows a distribution map of the "monofloral Erica", "Multifloral", "Virgin monofloral Erica" and "Virgin Multifloral" honey samples.

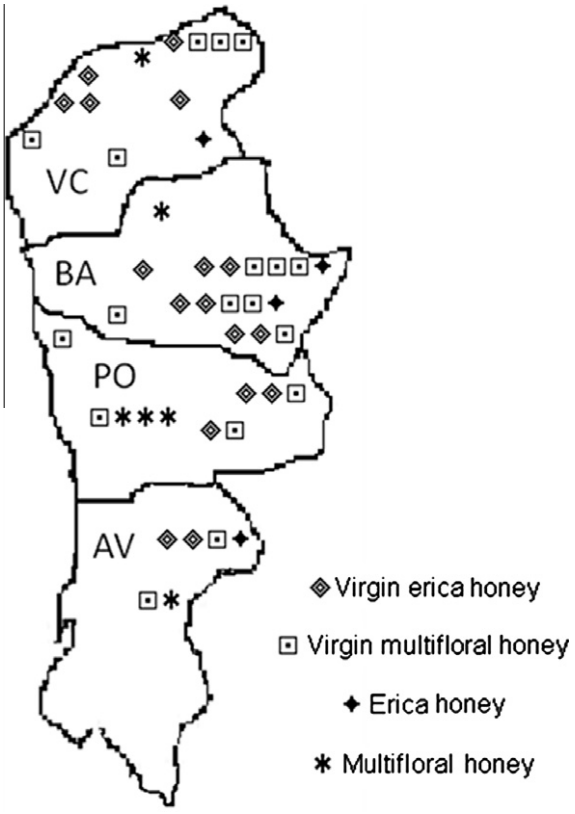

Fig. 2. Distribution of the honeys studied. AV (Aveiro), BA (Braga), PO (Porto) and VC (Viana do Castelo).

Ash and EC values depend on the mineral content of the honey: ash gives a direct measure of inorganic residue after carbonisation, while electric conductivity measures all ionizable organic and inorganic substances. The honeys considered in this study had ash contents ranging from 0.17% to 0.43%. Ash values were below 0.60%, as expected for nectar honeys (Codex Alimentarius, 2001; EU, 2002a).

The EC values of the honeys analyzed ranged from 0.46 to 0.99 mS/cm (mean value ± standard deviation = 0.66 ± 0.10 mS/cm). 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.

This model should replace the older and time consuming method for determining total ash mass fraction by ashing. Confirmation of this relationship, in the honeys analyzed, 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. The relation between EC and ash content has been demonstrated by many researchers who agree that the above-mentioned parameters are related (Felsner et al., 2004; Kropf et al., 2008). A model ($y = 1.74x + 0.14$) has been proposed for use all over Europe by the International Honey Commission (IHC) (Bogdanov et al., 1997). The present study found a linear correlation ($R = 0.997$) between the EC of honeys and their ash content,

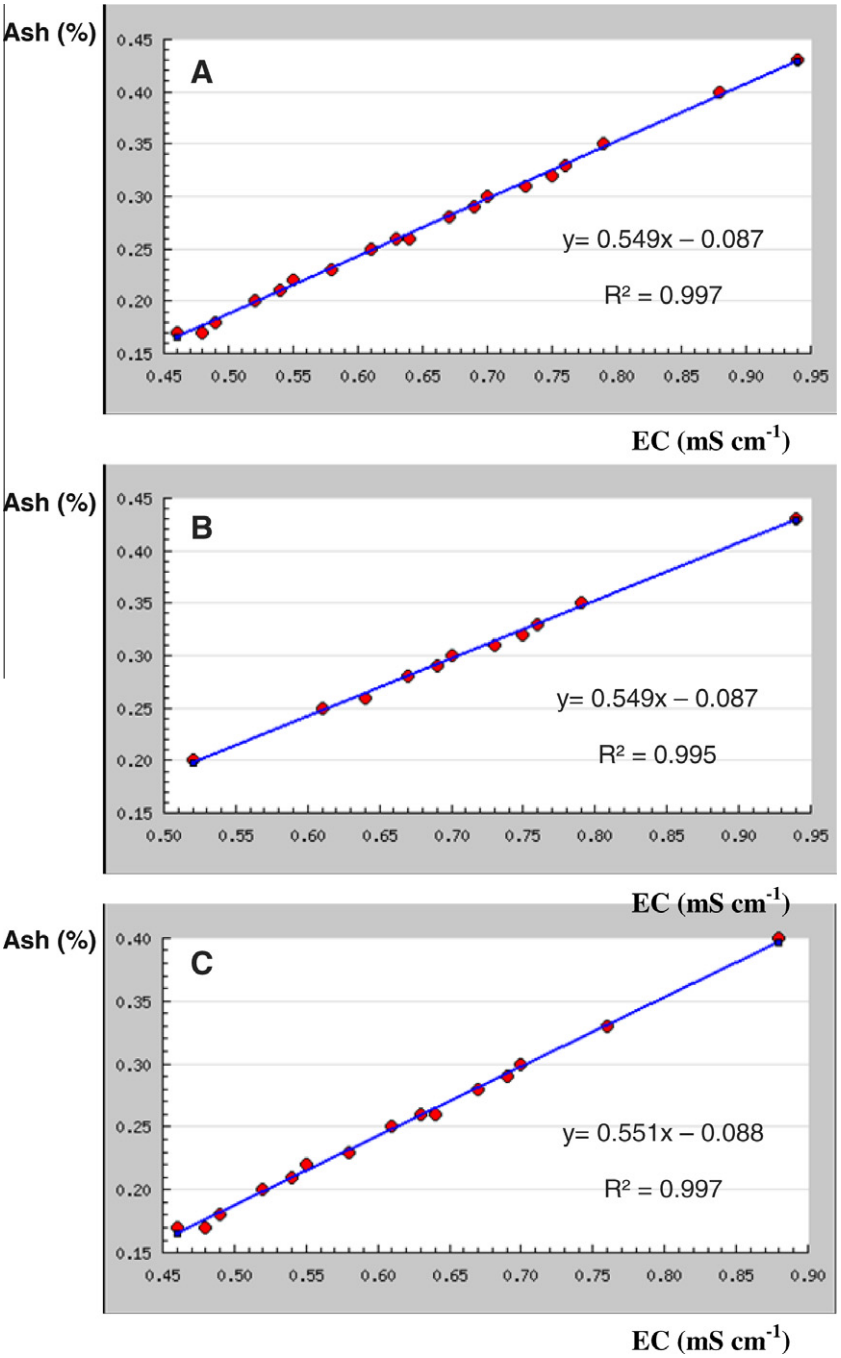

Fig. 3. Linear regression obtained between ash content and electrical conductivity for: (A) the total honey samples (B) monofloral *Erica* honeys and (C) multifloral honeys.

with a final regression model ($y = 0.549x + 0.087$), as presented in Fig. 3A. In the present work no significant differences between models for monofloral *Erica* honey (Fig. 3B) and multifloral honey (Fig. 3C) have been found. IHC linear regression is directly comparable to the model obtained here. In accordance with previous research, the linear regression model of ash mass fraction and EC is independent of honey type (Kropf et al., 2008).

The honey samples presented a pH from 3.5 to 4.2, with an average of 3.8. 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 its texture, stability and shelf life (Terrab et al., 2004). Published reports indicate that pH should be between 3.2 and 4.5. The values of pH in honey help to determine its origin: flower or forest; the latter show higher values (Bogdanov et al., 1997).

The FA of honey samples is 29.8 meq/kg (mean) with a range of 17.2–45.2 and a standard deviation of 5.8 meq/kg. Variation in FA among different honeys can be attributed to floral origin or to variation because of the harvest season (Perez-Arquillué et al., 1995). The FA 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 (Finola et al., 2007). All of the samples investigated met the demands set out in the regulations (Codex Alimentarius, 2001; EU, 2002a), which require in general not more than 50 meq/kg and not more than 80 meq/kg (baker's honey); this indicates the absence of unwanted fermentations.

Honey is mainly composed of the monosaccharides glucose and fructose. The RS (%) content of the honeys analyzed ranged from 66.7 to 76.9% (mean value ± standard deviation = 72.6 ± 2.3) and the mean percentages of AS is 3.7% with a range of 2.7–4.5 and a

Table 4
Microbial analyses of honey samples.

Sample	Aerobic mesophile (cfu/g)	Moulds and yeasts (cfu/g)	Fecal coliforms (MPN)	Sulphite-reducing Clostridia (in 0.01 g)	*Salmonella* (in 25 g)
1[a]	4.0×10^2	1.3×10	<1	Negative	Negative
2[b]	2.5×10^2	1.0×10	<1	Negative	Negative
3[a]	1.3×10^2	1.1×10	<1	Negative	Negative
4[a]	1.2×10^2	1.2×10	<1	Negative	Negative
5[b]	4.0×10^2	4.0×10	<1	Negative	Negative
6[b]	1.5×10^2	1.2×10	<1	Negative	Negative
7[a]	1.3×10^2	1.1×10	<1	Negative	Negative
8[b]	5.0×10^2	4.1×10	<1	Negative	Negative
9[b]	3.1×10^2	5.5×10	<1	Negative	Negative
10[b]	2.2×10^2	3.0×10	<1	Negative	Negative
11[a]	2.0×10^2	1.4×10	<1	Negative	Negative
12[a]	1.4×10^2	1.0×10	<1	Negative	Negative
13[a]	1.8×10^2	1.3×10	<1	Negative	Negative
14[b]	2.0×10^2	1.2×10	<1	Negative	Negative
15[b]	7.8×10^2	5.5×10	<1	Positive	Negative
16[a]	1.9×10^2	1.1×10	<1	Negative	Negative
17[a]	1.6×10^2	1.0×10	<1	Negative	Negative
18[a]	1.3×10^2	1.8×10	<1	Negative	Negative
19[a]	1.4×10^2	1.0×10	<1	Negative	Negative
20[a]	3.6×10^2	1.2×10	<1	Negative	Negative
21[b]	4.0×10^2	4.6×10	<1	Negative	Negative
22[b]	3.0×10^2	1.1×10	<1	Negative	Negative
23[b]	2.0×10^2	1.0×10	<1	Negative	Negative
24[b]	3.0×10^2	4.0×10	<1	Negative	Negative
25[b]	3.0×10^2	1.5×10	<1	Negative	Negative
26[b]	5.0×10^2	4.0×10	<1	Negative	Negative
27[b]	2.0×10^2	2.5×10	<1	Negative	Negative
28[a]	1.5×10^2	1.5×10	<1	Negative	Negative
29[b]	7.0×10^2	4.4×10	<1	Negative	Negative
30[a]	1.7×10^2	1.3×10	<1	Negative	Negative
31[b]	8.3×10^2	4.0×10	<1	Negative	Negative
32[a]	3.1×10^2	1.2×10	<1	Negative	Negative
33[b]	1.3×10^2	1.0×10	<1	Negative	Negative
34[b]	4.0×10^2	2.0×10	<1	Negative	Negative
35[a]	5.0×10^2	1.8×10	<1	Negative	Negative
36[b]	5.0×10^2	3.0×10	<1	Negative	Negative
37[b]	6.0×10^2	8.0×10	<1	Negative	Negative
38[a]	1.8×10^2	1.8×10	<1	Negative	Negative
39[a]	1.8×10^2	1.4×10	<1	Negative	Negative
40[b]	8.0×10^2	3.0×10	<1	Negative	Negative
41[a]	1.4×10^2	1.2×10	<1	Negative	Negative
42[b]	4.0×10^2	2.0×10	<1	Negative	Negative
43[a]	1.9×10^2	1.4×10	<1	Negative	Negative
44[a]	1.7×10^2	1.2×10	<1	Negative	Negative
45[b]	5.0×10^2	2.5×10	<1	Negative	Negative
mean	3.1×10^2	2.2×10	–	–	–
range ± SD	$(1.2 \times 10^2 - 8.3 \times 10^2) \pm 1.9 \times 10^2$	$(1.0 \times 10–8.0 \times 10) 1.6 \times 10$	–	–	–
[a]mean	2.0×10^2	1.3×10	–	–	–
[a]range ± SD	$(1.2 \times 10^2 - 5.0 \times 10^2) \pm 1.0 \times 10^2$	$(1.1 \times 10–1.8 \times 10) \pm 2.5$	–	–	–
[b]mean	4.1×10^2	3.1×10	–	–	–
[b]range ± SD	$(1.3 \times 10^2 - 8.3 \times 10^2) \pm 2.1 \times 10^2$	$(1.0 \times 10 - 8.0 \times 10) \pm 1.79 \times 10$	–	–	–

[a] Monofloral *Erica* samples found (*Erica* pollen percentage > 45%).
[b] Multifloral samples found.

standard deviation of 0.5 (sucrose content by European Directives must be under 5%). These two parameters confirm that the honey samples studied were floral honeys.

3.3. Microbial contaminations

The intrinsic properties of honey affect the growth and survival of microorganisms by bacteriostatic or bactericidal action, and particularly the low pH and high content of honey's sugars prevents the growth of many microorganisms (Iurlina and Fritz, 2005). Consequently, it is expected that honey contains a small number and limited variety of microorganisms. Table 4 shows the means, standard deviations and ranges of microbiological variables according to floral origin (Monofloral *Erica* honey/Multifloral honey).

Total yeast and moulds count in the sample ranged between 10 and 80 cfu/g, with the mean value 22.5. This indicates an appropriate management and the absence of unwanted fermentations. Aerobic mesophiles were detected in low count, with mean value obtained of 314. This might be the result of an adequate hygienic practice during harvesting and extraction of honey from the comby. Iurlina and Fritz (2005) found values similar to the obtained in this work, when analyzing in Argentinean's honeys. Although, these results were higher than the obtained by Gomes et al. (2010) in commercial Portuguese honeys.

In respect to fecal coliforms and *Salmonella*, all our samples were negative. In the other hand, Iurlina and Fritz (2005), detected coliform in a tested sample of Argentinean honey.

The count of sulfite-reducing Clostridia showed that 2% of the samples (1 out of 45) had this microorganism. These results were below the values obtained by Finola et al. (2007). These authors reported that 70% of 23 honey's samples were contaminated with this sulfite-reducing *Clostridia*. It is important to refer that the main source of *Clostridium* is the soil, but the equipment, dust, buildings and the environment play also an important role. The presence of sulfite-redutors *Clostridia*, indicates contamination or pollution. It might also indicate the existence of *C. botulinum*'s spores (Finola et al., 2007). The consumption of honey with *C. botulinum* spores is mainly dangerous to babies and kids, because in the absence of a competitive intestinal flora, and considering the high pH of their gut, the spores can germinate in the intestine and forming toxin in transit, causing infant botulism. These bacteria can also cause problems in immunosuppressed, and when the honey is therapeutically applied in wounds.

It was also found that the microbiological contamination of honey with *Erica* sp. was less than the multifloral honey's contamination. These results could be related with the content of phenolic compounds, because according Estevinho et al. (2008) and Ferreira et al. (2009), its amounts are higher in dark-colored honey, like Erica's honey. Indeed, many researchers attribute to these compounds high antimicrobial activity (Estevinho et al., 2008 and Oliveira et al., 2008).

4. Conclusion

From the economical standpoint, the assessment of floral origin, microbiological and physico-chemical properties may increase the commercial value of these artisanal honeys. In this work, the principal physico-chemical parameters, such as moisture, electrical conductivity, ash, hydroxymethylfurfural, diastase activity, pH, free acidity, reducing sugars and apparent sucrose, have been determined in 45 honeys from Northwest Portugal. All of the values obtained fell within the maximum limits defined under current international legislation (Standard Codex and EU). Furthermore, more than 77.8% of the samples should be labeled as "Virgin Honey" in accordance with EU rules. The melissopalynological method confirmed the identity of the flower sources visited by the bees. The present results indicated that 42% of the all the samples were monofloral *Erica sp*. honeys. All of the analyzed samples have an *Erica sp*. pollen percentage higher than 15%. Therefore, in the context of enhancing marketability of honeys, authorities, distributors and consumers should be provided with data composition. In addition, all the honey samples exceed the quality parameters and should be labeled as "virgin" honeys. This data would make artisanal honeys more attractive, at the same time that it would protect consumers against improper practices and also guarantee fair trade.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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