Characterization of Portuguese honey from Castelo Branco region according to their pollen spectrum, physicochemical characteristics and mineral contents

Luís R. Silva^{1,2} · Adriana Sousa^{1,2} · Marcos Taveira²

Revised: 9 May 2017 / Accepted: 12 May 2017 / Published online: 23 May 2017
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Abstract The aim of this work was to evaluate the quality of 16 honey samples from Castelo Branco region (Portugal). Twelve are monofloral from *Calluna vulgaris*, *Erica*, *Lavandula*, *Echium* and *Campanula*. The mean values obtained for physicochemical parameters were: 3.82 pH; 16.80% moisture; 81.6°Brix; 0.21% ash; 357.6 $\mu\text{S cm}^{-1}$ electrical conductivity; 33.7 meq/kg free acidity; 5.7 meq/kg lactic acid; 39.3 meq/kg total acidity; 9.11 mg/kg HMF; 21.3 IN invertase and 9.0° Gothe for diastase activities. The results indicate a good quality, adequate processing, good maturity and freshness of honey. Additionally, the determination of mineral contents revealed that the K was the major element. Mean values obtained were (mg/kg): Ca, 28.36; K, 701.87; Mg, 74.00; Na, 31.04; Fe, 0.97; Cu, 0.65; Zn, 1.23; Mn, 2.78 and P, 48.80. Among the overall determined parameters, CB2 stands out by its high values in minerals, pH, moisture, ash, electrical conductivity and enzyme activity.

Keywords Honey · Pollen analysis · Physicochemical analysis · Mineral content

Introduction

Honey is a sweet, viscous substance produced by *Apis mellifera* from the nectar of plants and secretion from plants or plant-sucking insects (Silva et al. 2009). The bees collect, transform and combine this material with specific substances of their own (enzymes), store and leave it to ripen in the honeycombs of beehive (European Community 2002). Honey is considered a desirable ingredient in a range of different foodstuffs, and it is appreciated by the consumers due its sweet taste, light flavor and attractive color. It can be used as food preservative, sugar substitute with the advantage of being a good source of bioactive compounds, and possesses valuable nourishing, healing and prophylactic properties (Babarinde et al. 2011; Escuredo et al. 2012; Alves et al. 2013).

Honey has been reported to contain about 200 substances, distributed by a complex mixture of sugars and small amounts of other constituents, such as proteins, minerals, vitamins, organic acids, aroma compounds, flavonoids, phenolic acids, enzymes, pigments, waxes, pollen grains and other phytochemicals (White 1979). It has been used in ethnomedicine in the treatment of burns, gastrointestinal disorders, asthma, skin ulcers, infected and chronic wounds, cataracts and other eye ailments (Al-Mamary et al. 2002).

The composition and sensory attributes of honey are influenced by several factors, such as botanical and geographical origin, climate, environmental conditions and the contribution of the beekeeper (White 1979; Anklam 1998), being the determination of botanical and geographical origin of honey of great interest worldwide. Furthermore, the diversity of the physical and chemical properties of honey depends on the pollen and nectar of the original plant, moisture, flavour, colour, contents of proteins and sugars (White 1979; Anklam 1998; Silva et al. 2009; Bettar et al. 2015).

✉ Luís R. Silva
luisfarmacognosia@gmail.pt

¹ CICS-UBI - Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior, Av. Infante D. Henrique, 6201-506 Covilhã, Portugal

² Instituto Politécnico de Castelo Branco, Escola Superior de Saúde Dr. Lopes Dias, Avenida do Empresário, Campus da Talagueira, 6000-767 Castelo Branco, Portugal

Quality parameters are useful to detect product adulteration during manufacture (water, sugar and syrup addition) and also to confirm a hygienic production and efficient storage of honey (Andrade et al. 1999). Control of honey requires the determination of parameters that could establish origin and calls for efforts to improve honey characterisation.

The classical approach to the classification of monofloral and multifloral honeys and the reliable determination of their botanical origin includes three complementary methods: sensory, melissopalynological and physicochemical analysis (European Community 2002). The analysis and identification of pollens grains contained in honey is usually determined by melissopalynology, the first technique to be used, and currently complemented by sensory analysis. However, the melissopalynological technique requires some expertise (Anklam 1998). Nonetheless, its known limitations have encouraged the search for physicochemical parameters as honey indicators. Several papers reported the physicochemical characterization of honeys (Singh and Bath 1997; Anklam 1998; Andrade et al. 1999; Bath and Singh 1999; Silva et al. 2009; Flores et al. 2015).

The contribution of minerals is relatively low and variable, and normally it accounts for 0.1–0.2% in floral honey and 1% or higher in mellate honeys (Hernández et al. 2005). The main concentrations of minerals derive from soil. They are transported to honey plants through the root system, pass to nectar and then to honey (Pohl 2009). The most abundant metal in honey is potassium (K), accounting for 45–85% of the total mineral profile. Other major inorganic species present in honey are sodium (Na), calcium (Ca) and magnesium (Mg). Honey contains also copper (Cu), iron (Fe), zinc (Zn) and manganese (Mnatzaganian et al. 2005) in intermediate quantities and trace metals at much lower levels ($<1 \mu\text{g g}^{-1}$) (Pohl 2009; Tuzen et al. 2007). The metal concentration in honey can be linked with floral type of honey plants, floral density, botanical origin of nectar and pollens and it can be used as an environmental indicator (Pohl 2009). Additionally, the mineral content of honey is one of the properties to be considered in the evaluation of its nutritional value and has been studied by several authors (Hernández et al. 2005; Pohl 2009; Silva et al. 2009; Alves et al. 2013).

The demand for classifying the geographical and botanical origins of honey is required. All countries need to specify floral origin, source, organoleptic characteristics, phytochemical properties and local origin (Anklam 1998). This practice assures that the properties, quality and the origin of honey in the market are in accordance with the information stated on the label.

The main purpose of this work was to determine the pollen composition, evaluate the physicochemical properties (pH, free, lactic and total acidity, electrical

conductivity, moisture content, hydroxymethylfurfural, diastase and invertase activities) and mineral contents (K, Na, Ca, Mg, Fe, Cu, Zn, Mn and P) of 16 honey samples from different botanical origins and representative of the Castelo Branco region (Portugal).

Materials and methods

Sampling

Sixteen honey samples were collected in Castelo Branco district (Portugal). Sampling area covered the most important production zones (Table 1). They were collected during the year 2015. Samples were stored in a dry and dark place at 20 °C until analysis, which occurred no longer than two months after extraction from the hives by beekeepers.

Pollen analysis

The study of the honey pollen spectra was carried out in accordance to the melissopalynological method described before (Louveau et al. 1978). Honey (10 g) was diluted with bidistilled water (50 mL) and centrifuged at 4500 rpm for 10 min. The obtained sediment was dissolved in water and centrifuged for 5 min. Two aliquots of 100 μL of the sediment were used for the determination of the pollen spectrum: a minimum of 800 pollen grains were examined microscopically at 45 $\times$, using a bright-field microscope (Olympus, Tokyo). The results were expressed as percentages.

Physicochemical characteristics

The physicochemical characteristics were performed according to Silva et al. (2009). All assays were performed in triplicate.

pH

The pH was evaluated in a solution of 10 g of honey in 75 mL of distilled water, using a HI 110 Benchtop pH Meter with custom buffers (HANNA instruments, Italy).

Moisture content

Moisture was measured by refractometry at 25 °C with an Atago (IT Abbe, Japan) refractometer.

Ash

To determine the ash content, honey samples were calcined overnight, in furnace at 550 °C (Model 3216P1, Lenton, UK), until constant mass.

Table 1 Relative content of different pollen types present in honeys from Castelo Branco (Portugal)

Sample	D (> 45%)	A (15–45%)	I (3–15%)	R (1–3%)
CB1	<i>Erica</i> spp. (51%)		<i>Eucalyptus</i> spp. (9.0%) <i>Cytisus</i> spp. (7.3%) <i>Rubus</i> spp. (4.7%) <i>Echium</i> (4.5%) <i>Castanea sativa</i> (4.2%) <i>Cistus ladanifer</i> (3.9%) <i>Cistacea</i> (3.5%)	<i>Lavandula</i> spp. (2.8%) <i>Scandix</i> type (2.5%) Honeydew elements (2.2%)
CB2	<i>Calluna vulgaris</i> (66.3%)		<i>Cytisus</i> spp. (9.8%) <i>Rubus</i> spp. (5.1%) <i>Castanea sativa</i> (3.7%) <i>Cistus ladanifer</i> (3.7%) <i>Cistacea</i> (3.1%)	<i>Lavandula</i> spp. (1.8%) Honeydew elements (1.8%) <i>Anthemis arvenses</i> (1.2%)
CB3		<i>Erica</i> sp. (29.6%) <i>Lavandula</i> type (19.2%) <i>Cistus ladanifer</i> (16.0%)	<i>Cytisus</i> type (14.4%) <i>Rubus</i> (9.6%) <i>Eucalyptus</i> spp. (3.2%)	<i>Raphanus</i> spp. (1.6%)
CB4	<i>Echium</i> (66.1%)	<i>Lavandula</i> spp. (18.3%) <i>Erica</i> sp. (4.6%)		<i>Cistus ladanifer</i> (2.8%) <i>Rubus</i> (1.8%)
CB5		<i>Erica</i> sp. (38.0%) <i>Citrus</i> spp. (20.7%) <i>Lavandula</i> spp. (16.5%)	<i>Echium</i> (5.0%) <i>Lotus</i> (5.0%)	<i>Cistacea</i> (7.4%) <i>Frangula</i> spp. (1.7%) <i>Cytisus</i> type (1.7%)
CB6		<i>Trifolium</i> type (36.8%) <i>Poaceae</i> (24.1%) <i>Thymus</i> type (15.5%)	<i>Eucalyptus</i> spp. (8.0%) <i>Anthemis arvensis</i> (4.6%)	<i>Scandix</i> type (2.9%) <i>Echium</i> (2.3%) <i>Erica</i> sp. (1.7%) <i>Calendula arvensis</i> (1.7%) <i>Lavandula</i> spp. (1.1%) <i>Rubus</i> (1.1%)
CB7		<i>Echium</i> (33.1%) <i>Raphanus</i> spp. (19.8%)	<i>Trifolium</i> type (12.4%) <i>Cistus ladanifer</i> (10.7%) <i>Rhamnus</i> type (5.8%) <i>Eucalyptus</i> spp. (4.1%) * <i>Rubus</i> (3.3%)	<i>Carduus</i> type (2.5%) <i>Lavandula</i> spp. (2.5%) <i>Frangula</i> spp. (2.6%) <i>Hypericum</i> spp. (2.6%) <i>Salix</i> spp. (2.6%)
CB8		<i>Echium</i> (31.3%) <i>Erica</i> spp. (19.4%)	<i>Rubus</i> spp. (12.7%) <i>Lavandula</i> spp. (12.3%) <i>Cistus ladanifer</i> (7.8%) <i>Campanula</i> spp. (5.1%) <i>Eucalyptus</i> spp. (4.3%)	<i>Acacia</i> spp. (2.8%) <i>Cytisus</i> spp. (2.2%) <i>Trifolium</i> spp. (1.1%)
CB9		<i>Echium</i> (26.3%) <i>Acacia</i> spp. (19.3%) <i>Lavandula</i> spp. (16.7%)	<i>Rhamnus</i> type (10.5%) <i>Erica</i> sp. (4.4%) <i>Campanula</i> sp. (3.5%) <i>Cytisus</i> type (3.5%)	<i>Castanea sativa</i> (1.8%) <i>Reseda</i> spp. (1.8%)
CB10	<i>Echium</i> (70.0%)		<i>Lavandula</i> spp. (10.8%) <i>Cistus ladanifer</i> (5.4%) <i>Acacia</i> spp. (4.2%)	<i>Rubus</i> (3.0%) <i>Eucalyptus</i> spp. (1.8%) <i>Cytisus</i> spp. (1.2%) <i>Trifolium</i> spp. (1.2%)

Table 1 continued

Sample	D (> 45%)	A (15–45%)	I (3–15%)	R (1–3%)
CB11		<i>Lavandula</i> spp. (21.7%)	<i>Echium</i> (14.6%)	<i>Cistacea</i> (2.4%)
		<i>Rubus</i> (18.9%)	<i>Campanula</i> sp. (11.8%)	<i>Cistus ladanifer</i> (1.9%)
			<i>Cytisus</i> spp. (9.9%)	<i>Frangula</i> spp. (1.4%)
			<i>Acacia</i> spp. (5.2%)	
			Honeydew elements (4.2%)	
			<i>Eucalyptus</i> spp. (3.8%)	
CB12		<i>Echium</i> (34.0%)	<i>Salix</i> spp. (8.6%)	<i>Cytisus</i> spp. (2.9%)
		<i>Olea</i> (30.6%)	<i>Cistus ladanifer</i> (7.2%)	<i>Eucalyptus</i> spp. (1.9%)
			<i>Campanula</i> spp. (5.7%)	
			Honeydew elements (3.8%)	
			<i>Lavandula</i> spp. (3.8%)	
CB13		<i>Cistus ladanifer</i> (32.3%)	<i>Cistacea</i> (9.7%)	<i>Frangula</i> spp. (1.9%)
		<i>Lavandula</i> spp. (16.8%)	<i>Rubus</i> spp. (9.7%)	<i>Raphanus</i> spp. (1.9%)
			<i>Cytisus</i> spp. (8.4%)	<i>Acacia</i> spp. (1.3%)
			<i>Erica</i> spp. (7.1%)	<i>Olea</i> (1.3%)
			<i>Echium</i> (5.2%)	
			<i>Quercus</i> spp. (3.2%)	
CB14	<i>Campanula</i> spp. (47.0%)	<i>Rubus</i> (37.1%)	<i>Echium</i> (4.0%)	<i>Olea</i> (2.6%)
			<i>Eucalyptus</i> spp. (3.3%)	<i>Lavandula</i> spp. (2.0%)
				<i>Frangula</i> spp. (1.3%)
CB15	<i>Erica</i> spp. (66.4%)	<i>Rubus</i> (15.1%)	<i>Cytisus</i> spp. (11.8%)	<i>Cistus ladanifer</i> (2.6%)
				Honeydew elements (2.6%)
				<i>Eucalyptus</i> spp. (1.3%)
CB16		<i>Lavandula</i> spp. (27.8%)	<i>Cistus ladanifer</i> (14.2%)	<i>Eucalyptus</i> spp. (2.8%)
		<i>Erica</i> spp. (16.5%)	<i>Raphanus</i> spp. (9.1%)	<i>Cistacea</i> (1.7%)
			Honeydew elements (8.5%)	<i>Pinus</i> spp. (1.1%)
			<i>Cytisus</i> spp. (6.8%)	<i>Echium</i> (1.1%)
			<i>Rubus</i> (6.25%)	<i>Acacia</i> spp. (1.1%)

D, predominant pollen (> 45%); A, accompanying pollen (15–45%), I, important pollen (3–15%); R, minor pollen (1–3%)

Electrical conductivity

The electrical conductivity was measured in honey solution at 20% (dry matter basis) in distilled water at 20 °C in a conductimeter (HI 9033, HANNA instruments, Italy).

Sugar

Sugar content was evaluated by refractometry, and the results were expressed as °Brix.

Free, lactonic, and total acidity

Free, lactonic and total acidity were determined by titrimetric method (Silva et al. 2009). Free acidity: 0.05 M NaOH was added until pH 8.50. Lactonic acidity: immediately following the free acidity, a volume of 10 mL 0.05 M NaOH was added and, without delay, back-titrated

with 0.05 M HCl to pH 8.30. Finally, the total acidity was determined by the sum of free and lactonic acidities.

Hydroxymethylfurfural content (HMF)

The HMF of honey samples was determined using the Winkler method (Silva et al. 2009): 5 g of each sample were treated with a clarifying agent (Carrez), the volume was completed to 50 mL and the solution was filtered. The absorbance was measured at 284 and 336 nm against an aliquot treated with NaHSO₃.

Diastase activity

Diastase activity was determined using a starch solution and honey heated in thermostatic bath at 40 °C (Silva et al. 2009). Absorption was measured using a UV/VIS double beam spectrophotometer (U-2900/2910, Hitachi, Japan)

and a chronometer. Using regression (without using the data point at 0 min), lines were fitted to the absorption data and the diastase number was calculated from the time taken for the absorbance to reach 0.235. Results were expressed in Gothe degrees as mL of 1% starch hydrolysed by enzyme in 1 g of honey, during 1 h.

Invertase activity

Invertase was determined using the Siegenthaler method (Siegenthaler 1977), harmonised by the European Honey Commission (Bogdanov et al. 1997). The enzyme activity is evaluated photometrically, by measuring the decomposition of the substrate *p*-nitrophenyl α -D glucopyranoside into the product *p*-nitrophenol (which has a maximum absorbance at 400 nm). The results were expressed as invertase number (IN). The IN indicates the amount of sucrose per gram hydrolysed in 1 h by the enzymes contained in 100 g of honey under test conditions.

Determination of mineral elements

The ash contents of honey samples were determined by calcination at 550 °C of 5 g, until constant mass. A volume of five millilitres of nitric acid 0.1 M were added to the resultant ashes. The obtained mixture was stirred on a heating plate until complete dryness. Subsequently, 10 mL of the nitric acid were added and the mixture was completed to 25 mL with distilled water. Minerals were determined by atomic absorption spectrometry (Perkin Elmer, Analyst 300), using an air/acetylene flame. Quantitative determination of the elements was carried out after calibrating the instrument, using Ca, K, Mg, Na, Fe, Cu, Zn, Mn and P solutions dissolved in 0.1% lanthanum (La). La was used as a matrix modifier in order to overcome the chemical interferences in the air/acetylene flame. All samples were analysed in triplicate.

Statistical analysis

Principal component analysis (PCA) was carried out using XLSTAT 2016.1 software. The PCA method shows similarities between samples projected on a plane and makes it possible to identify which variables determine these similarities and in what way.

Results and discussion

Several types of honey are produced in Portugal, where honey production is a traditional practice well implanted in several regions. The detailed characterization of the different honey types existent in Portugal is important, since it

will allow the establishment of technical specifications avoiding of adulterations. In addition, chemical composition influences the sensorial characteristics, being important for commercial aspects and health benefit effects associated with the honey consumption.

Pollen analysis

Knowledge about the pollen morphology of honey plants is important in the identification of plant species which contribute to honey's composition. Pollen analysis can provide important data about honey characterization, concerning its geographic origin, collection season, occurrence of nectariferous plants, and at a later time honey determination from an unknown or questionable origin. Castelo Branco region is located in the south centre of Portugal, being an important region of honey production, associated with its specific edafoclimatic conditions and wide plant diversity, which is corroborated by the fact that 25% of the honey samples analysed are multifloral, being composed by under-represented pollen (Table 1). Thirty pollen types were detected in the studied samples (Fig. 1 and Table 1). Pollen from *Lavandula* (93.75%) and *Echium* (81.25%) were the most frequent. Other frequent pollen types were from *Rubus* (68.75%), *Cytisus* (68.75%), *Cistus ladanifer* (68.75%), *Eucaliptus* (62.5%) and *Erica* (62.5%) (Table 1 and Fig. 1).

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. For honey samples having under-represented pollen grains, botanical classification may be achieved with a minor pollen frequency

Fig. 1 Frequency of the different pollen types in the studied samples

percentage, as for example, lavender honey needs 15% of *Lavandula* sp. pollen to be monofloral. The twelve monofloral honeys identified are divided into *Erica* spp. (CB1 and CB15), *Calluna vulgaris* (CB2), *Lavandula* sp. (CB3, CB5, CB9, CB11, CB 13 and CB16), *Echium* spp. (CB4 and CB10) and *Campanula* (CB14). On the other hand, multifloral honeys contained several pollen types with a considerable percentage of pollen grains from *Erica* spp., *Echium* spp., *Campanula* spp., *Cistus ladanifer* and *Lavandula* spp. (Table 1). The pollen analysis of the honey from Castelo Branco is reported for the first time. Previous studies on Portuguese pollen of honey from Serra da Lousã were performed in 60 honey samples, 70% were monofloral honeys from *Erica* sp., 17% monofloral honeys from Ericaceae (*Erica* sp. and *Calluna vulgaris*) and 13% multifloral honeys with a high percentage of *Erica* sp (Andrade et al. 1999). In another study with 38 honey samples from Luso region, the pollen analysis revealed the presence of considerable percentage of *Eucalyptus* sp., *Erica* sp., *Rubus* sp., *Lavandula stoechas*, *Castanea sativa* and *C. scoparius* (Silva et al. 2009). Estevinho et al. (2012) performed a study with 75 honey samples from Trás-os-Montes region noting that all samples were classified as monofloral *Erica* sp., with important levels of pollen grains of *Echium* sp., *Castanea* sp., *Lavandula* sp., *Cistus* sp. and *Rubus* sp.

In Europe there exists more than 100 botanical species that can give unifloral honeys, great part of them is important in their production region and are thus marketed on a limited scale, but they have been described in the literature (Bogdanov et al. 2004). Lavender honey is produced in Portugal by *Lavandula* sp. Lavender is a popular aromatic herb from Mediterranean region belonging to Lamiaceae family. The genus *Lavandula* contains several species, the most common species that are believed to have aromatherapy and pharmaceutical value are *Lavandula dentata*, *L. angustifolia*, *L. latifolia*, *L. stoechas* and *L. Hybrid* (Castro-Vázquez et al. 2014). This honey is characterized by their “spike lavender”, being currently among the most appreciated high-quality honeys due to its pleasant aroma and taste. Additionally, *Echium plantagineum* L. is also native from Southern Europe, and is characterized by its flower with a purple corolla and two protruding stamens and one stigma. This plant produces typical dark blue pollen, which is collected by bees and commercialized (Moita et al. 2013).

Physicochemical parameters

The obtained physicochemical parameters are shown in Table 2. The pH of honey is important for its texture,

stability and shelf-life and can be affected by the storage process and extraction conditions. The analysed samples revealed pH values between 3.34 and 5.33 (mean value = 3.82). These values are similar to those obtained with other Portuguese honeys (Andrade et al. 1999; Silva et al. 2009) and honeys from *Trifolium* spp., *Brassica juncea*, *Helianthus annuus* and *Eucalyptus lanceolatus* (Singh and Bath 1997; Bath and Singh 1999).

The harvesting season, the degree of maturity and climatic factors, as well as the botanical origin, can influence the water content of honey, which is regulated for safety reasons to avoid fermentation (Babarinde et al. 2011). High moisture content in honey is associated with yeast fermentation during storage, leading to a higher acidity level. All samples presented less than 20% water, the maximum content allowed by both Portuguese and international legislations (Decreto-lei N° 214/2003), except sample 2, which presented 24% of water content. Similar values were found in previous works (Bath and Singh 1999; Silva et al. 2009).

The values of Brix degrees ranged from 73.8 to 83.4 °Brix, being comparable to those from other Portuguese honey samples (Andrade et al. 1999; Silva et al. 2009) and honeys from other countries (Singh and Bath 1997; Bath and Singh 1999). These values are directly related with sugar content and anomalous values may be correlated with honey adulteration (Conti 2000; Terrab et al. 2004).

The ash content results (Table 2) are within the limit allowed for floral honeys (0.6%), indicating clearness of honey samples (Mendes et al. 1998). This parameter can be also used to determine the botanical origin (floral, mix or honeydew) (White 1978).

The electrical conductivity is closely related to the concentration of mineral salts, organic acids and proteins, and it is associated with the floral origin, being used as a honey quality indicator, assisting in the identification and distinction of floral honey and honeydew (Babarinde et al. 2011, Terrab et al. 2002; Bettar et al. 2015). Honey samples with electrical conductivity values lower than 800 $\mu\text{S cm}^{-1}$ are considered blossom honey or a blend of blossom with honeydew honey, and honey samples with higher values are considered honeydew honey (Bogdanov 2007). The results obtained for electrical conductivity varied between 130.2 and 667.4 $\mu\text{S cm}^{-1}$, all of them below the maximum limit indicated by Portuguese legislation (800 $\mu\text{S cm}^{-1}$), and all of them were considered blossom honeys. In a general way, high correlation was found between ash and electrical conductivity (data not shown).

The acidity of honey is associated with the presence of organic acids and inorganic ions (Echigo and Takenaka

Table 2 Distribution data for physicochemical parameters in Castelo Branco (Portugal) honey samples

Sample	pH	Moisture (%)	Ash (%)	El. conductivity ($\mu\text{S cm}^{-1}$)	Brix (%)	Free acidity (meq/kg)	Lactic acid (meq/kg)	Total acidity (meq/kg)	HMF (mg/kg)	Diastase activity ($^{\circ}$ Gothe)	Invertase activity (IN)
CB1	3.67 (0.02)	16.77 (0.06)	0.02 (0.00)	130.2 (21.0)	81.8 (0.00)	24.5 (1.20)	6.5 (0.87)	31.0 (0.87)	7.80 (0.29)	8.1 (0.34)	18.0 (0.62)
CB2	5.33 (0.05)	24.20 (0.00)	0.46 (0.01)	663.1 (10.3)	73.8 (0.10)	14.8 (0.76)	4.8 (0.76)	19.6 (0.29)	12.19 (0.74)	15.8 (0.61)	24.2 (0.82)
CB3	4.13 (0.01)	17.10 (0.00)	0.23 (0.00)	360.1 (11.2)	81.4 (0.00)	41.1 (0.58)	6.7 (1.08)	47.8 (1.53)	14.44 (0.67)	9.0 (0.32)	15.8 (0.54)
CB4	4.29 (0.00)	17.50 (0.01)	0.22 (0.00)	377.1 (12.5)	81.2 (0.00)	43.3 (0.55)	6.7 (0.76)	50.0 (0.50)	21.21 (0.90)	10.9 (0.53)	20.0 (0.49)
CB5	4.29 (0.00)	17.37 (0.06)	0.29 (0.00)	463.0 (18.4)	81.2 (0.00)	42.5 (0.25)	7.2 (0.29)	49.7 (0.29)	14.06 (0.62)	11.6 (0.61)	25.8 (0.53)
CB6	3.85 (0.05)	17.63 (0.05)	0.19 (0.00)	335.3 (30.0)	80.8 (0.00)	32.1 (0.28)	7.2 (1.76)	39.3 (1.54)	11.35 (0.56)	13.1 (0.26)	25.5 (0.40)
CB7	4.64 (0.05)	17.63 (0.04)	0.45 (0.01)	650.4 (40.3)	80.9 (0.12)	28.6 (0.74)	2.3 (0.25)	30.9 (0.74)	11.36 (0.82)	12.5 (0.39)	33.8 (0.73)
CB8	3.34 (0.16)	17.43 (0.06)	0.05 (0.00)	175.1 (9.15)	81.1 (0.10)	22.5 (1.00)	4.2 (0.38)	26.7 (1.38)	6.20 (0.33)	6.2 (0.38)	19.9 (0.54)
CB9	4.29 (0.03)	17.17 (0.21)	0.15 (0.00)	285.2 (21.2)	81.6 (0.00)	26.8 (0.30)	3.9 (0.13)	30.7 (0.38)	8.02 (0.62)	6.1 (0.30)	17.1 (0.62)
CB10	3.86 (0.02)	15.53 (0.10)	0.03 (0.00)	145.1 (13.4)	82.9 (0.00)	16.6 (0.13)	3.7 (0.38)	20.3 (0.30)	4.15 (0.21)	5.2 (0.21)	12.8 (0.46)
CB11	3.92 (0.02)	17.03 (0.12)	0.22 (0.00)	370.3 (12.3)	81.8 (0.00)	45.0 (1.20)	5.3 (0.29)	50.3 (1.07)	9.28 (0.48)	7.9 (0.31)	15.2 (0.89)
CB12	4.60 (0.01)	15.60 (0.03)	0.41 (0.00)	597.3 (32.3)	83.4 (0.00)	44.0 (0.50)	5.2 (0.65)	49.2 (1.99)	13.23 (0.54)	13.3 (0.42)	37.4 (0.41)
CB13	4.13 (0.01)	17.20 (0.04)	0.14 (0.00)	277.1 (17.1)	81.8 (0.00)	27.0 (0.30)	4.1 (0.32)	31.1 (0.32)	10.94 (0.58)	8.1 (0.46)	21.6 (0.41)
CB14	3.75 (0.01)	16.80 (0.08)	0.31 (0.00)	480.0 (9.04)	81.2 (0.00)	41.6 (0.50)	5.2 (1.25)	46.8 (0.30)	25.45 (1.10)	8.9 (0.37)	29.3 (1.22)
CB15	4.26 (0.07)	17.20 (0.12)	0.46 (0.01)	667.4 (37.4)	81.2 (0.06)	45.0 (0.60)	2.5 (0.05)	47.5 (0.06)	15.91 (0.60)	10.7 (0.32)	16.0 (0.77)
CB16	3.96 (0.02)	16.80 (0.09)	0.39 (0.01)	585.0 (20.3)	81.4 (0.06)	42.8 (0.90)	4.8 (0.50)	47.6 (0.05)	10.43 (0.63)	9.9 (0.49)	24.5 (0.71)
Mean	3.82	16.80	0.21	357.6	81.6	33.7	5.7	39.3	9.11	9.0	21.3
SD	0.21	0.02	0.26	321.6	0.3	12.9	1.2	11.7	1.86	1.3	4.6
Minimum	3.34	15.53	0.02	130.2	73.8	14.8	2.5	19.6	4.15	5.2	12.8
Maximum	5.33	24.20	0.46	667.4	83.4	45.0	7.2	50.0	25.45	15.8	37.4

SD standard deviation

1974; Terrab et al. 2004) and is considered the reserve of acidity when the honey becomes alkaline, while the total acidity is the sum of free and lactic acidities (Terrab et al. 2002). This measurement has several uses such as evaluating honey fermentation, authentication of unifloral honeys and differentiating nectar from honeydew honeys (Nanda et al. 2003). The free acidity was within the Portuguese and European legislations allowed limits (below 50 meq/kg), indicating that the honey samples did not undergo undesirable fermentations and are still fresh. The values observed for lactic acidity ranged from 2.5 to 7.2 meq/kg and the total acidity varied between 19.6 and 50.0 meq/kg. These variations can be attributed to the harvest season (Rodríguez et al. 2004). These results are in accordance with previously reported data for other Portuguese honeys (Mendes et al. 1998; Andrade et al. 1999) as well as for samples collected from other sites (Singh and Bath 1997; Bath and Singh 1999; Costa et al. 1999; Terrab et al. 2004; Bettar et al. 2015).

Honey freshness is generally evaluated by determining the parameters that increase or decrease with overheating and/or ageing, being the HMF, diastase and invertase the most common parameters.

HMF and diastase are included as international quality standards for honey. The first is virtually absent in fresh honey, produced only during acid-catalysed dehydration of hexoses, such as fructose or glucose, and it can be affected by several factors, such as storage conditions and floral sources (Costa et al. 1999; Terrab et al. 2004; Bettar et al. 2015). All the samples showed HMF contents below 25.45 mg/kg of honey, being within the European legislation, corresponding to a high degree of freshness. The diastase activity is especially susceptible to heating so it can be used as a parameter to determine if honey has been extensively heated during processing. Four samples presented an inappropriate diastase activity (Table 2), with values below 8° Gothe (Decreto-lei N° 214/2003), suggesting inadequate storage or processing.

Invertase (sacharase, α -glucosidase) is one of the most important honey enzymes, being a glycoprotein originating from hypopharyngeal glands of bees that converts sucrose into saccharides—glucose and fructose, which are physiologically usable by bees. Invertase activity is used as a parameter related to the freshness of honey, to its warming or storage conditions, being proposed by the European Honey Commission as a criterion of the quality of honey (Bogdanov et al. 1997) and, for this reason; the determination of this enzyme was taken into account. A wide range of activities was found in the analysed honey samples (from 12.8 to 37.4 IN), with an average of 21.3 IN (Table 2).

Mineral contents

The contribution of minerals is relatively low and normally accounts for 0.1–0.2% of nectar honeys, however the total mineral content of honeydew can exceed 1% (Hernández et al. 2005). The mineral content can be correlated with environmental pollution and is a potential indicator of geographical origin of honey, as the main concentrations of minerals in honey derive from soil (Anklam 1998). The results of the mineral contents are presented in Table 3. The samples CB2 (2786.48 mg/kg) and CB12 (1775.80 mg/kg) were found to be richer in minerals, and CB8 (101.76 mg/kg) and CB10 (50.40 mg/kg) were the poorest ones (Table 3). Potassium was the major mineral in all analysed samples, accounting for 67.7–85.3% of the total mineral profile, with an average of 701.87 ppm (Table 3); these results are in accordance with previous works, reporting contents between 45 and 85% (Pohl 2009; Silva et al. 2009, 2012). Magnesium, phosphorous, sodium and calcium were also present in moderate amounts in the samples, with average levels of 74.0, 48.8, 31.0 and 28.4 ppm, respectively (Table 3). The other minerals (iron, copper, zinc and manganese) were found in residual levels. The mineral contents are similar to those described previously (Terrab et al. 2003; Downey et al. 2005; Silva et al. 2009).

PCA analysis

To assess the variation of physicochemical characteristics and mineral contents of honey samples, PCA was performed on the obtained data. Figure 2 shows the projection of physicochemical characteristics and minerals of all honey samples into the plane composed by principal axes F1 and F2 containing 73.55% of the total variance. CB3 and CB11 are projected into the plane formed by F1 negative axis and F2 positive axis due to their high lactic acidity and brix. The honey samples CB4, CB5, CB12, CB15 and CB16 are projected in the plane formed by F1 and F2 positive axis, while CB1, CB6, CB8, CB9, CB10 and CB13 are projected into F1 and F2 negative axis. This separation may be due to the higher contents in P and Mg, and higher total acidity, free acidity, ash, electrical conductivity, HMF and invertase found in the samples projected into F1 and F2 positive axis. Additionally, CB2 and CB7 honey samples are projected into the plane formed by F1 positive axis and F2 negative axis due their high contents in Ca, K and Na and high values of moisture, pH and diastase, being the difference more pronounced for the CB2 sample, justifying its distancing from all other honey samples.

Table 3 Mineral content in Castelo Branco (Portugal) honey samples (mg/kg)

Sample	Ca	K	Mg	Na	Fe	Cu	Zn	Mn	P	Total
CB1	9.05 (0.53)	103.49 (2.56)	5.33 (0.19)	8.19 (0.53)	0.18 (0.05)	0.02 (0.00)	0.73 (0.05)	0.18 (0.02)	25.55 (1.28)	152.72
CB2	234.63 (7.60)	2343.9 (27.28)	67.79 (1.61)	82.07 (4.59)	0.85 (0.20)	0.58 (0.03)	0.56 (0.01)	22.62 (1.63)	33.48 (2.56)	2786.48
CB3	22.55 (1.05)	687.62 (10.09)	80.50 (3.04)	28.03 (0.57)	0.78 (0.05)	0.40 (0.05)	1.04 (0.02)	2.23 (0.20)	55.00 (1.00)	878.15
CB4	14.29 (0.68)	641.71 (7.88)	80.45 (3.02)	10.09 (0.38)	1.16 (0.14)	0.18 (0.02)	1.36 (0.09)	0.98 (0.09)	54.42 (2.26)	804.64
CB5	16.97 (1.52)	1048.10 (5.31)	107.82 (3.91)	28.95 (1.14)	1.57 (0.21)	0.21 (0.03)	1.62 (0.11)	0.80 (0.06)	79.45 (3.68)	1285.49
CB6	19.13 (0.26)	342.23 (7.50)	2.13 (0.07)	41.07 (2.00)	0.33 (0.05)	0.02 (0.00)	0.57 (0.03)	nq	36.12 (1.00)	441.6
CB7	21.29 (1.01)	1170.80 (15.19)	129.88 (2.28)	32.05 (3.23)	0.96 (0.10)	0.98 (0.01)	1.02 (0.05)	6.33 (0.25)	39.09 (1.05)	1402.4
CB8	13.82 (1.11)	44.42 (2.73)	3.65 (0.15)	21.42 (1.03)	0.63 (0.17)	nq	0.34 (0.05)	nq	17.48 (0.50)	101.76
CB9	9.64 (1.02)	309.90 (10.00)	34.10 (2.67)	13.99 (0.57)	0.27 (0.10)	0.08 (0.01)	0.80 (0.10)	0.81 (0.08)	34.74 (2.01)	404.33
CB10	2.77 (0.14)	42.98 (0.17)	1.79 (0.25)	0.36 (0.03)	nq	nq	0.03 (0.00)	nq	2.47 (0.15)	50.40
CB11	13.06 (0.50)	446.01 (5.03)	101.77 (1.09)	18.87 (0.99)	0.53 (0.05)	0.16 (0.01)	1.86 (0.16)	0.90 (0.05)	39.12 (2.51)	622.28
CB12	21.28 (0.52)	1352.90 (32.78)	230.81 (6.64)	40.36 (2.01)	2.52 (0.16)	1.36 (0.09)	3.29 (0.15)	4.05 (0.20)	119.23 (1.51)	1775.80
CB13	15.28 (0.25)	594.31 (5.02)	71.97 (1.15)	19.53 (0.50)	0.74 (0.10)	0.31 (0.08)	1.37 (0.05)	1.62 (0.15)	50.95 (2.66)	756.08
CB14	24.58 (1.03)	653.11 (15.04)	68.01 (2.52)	32.79 (1.90)	1.44 (0.05)	0.63 (0.05)	2.03 (0.22)	1.32 (0.06)	90.48 (1.50)	874.39
CB15	3.76 (0.16)	971.25 (25.09)	128.29 (4.55)	95.13 (33.59)	2.68 (0.05)	5.35 (0.30)	2.14 (0.10)	1.06 (0.14)	64.57 (2.32)	1274.23
CB16	11.70 (1.08)	477.24 (2.50)	69.69 (2.80)	23.78 (2.25)	0.93 (0.10)	0.12 (0.01)	0.95 (0.13)	1.51 (0.06)	38.57 (1.62)	624.49
Mean	28.36	701.87	74.00	31.04	0.97	0.65	1.23	2.78	48.80	
SD	55.37	587.28	60.21	25.21	0.77	1.31	0.82	5.54	28.88	
Minimum	2.77	42.98	1.79	0.36	0.18	0.00	0.03	0.00	2.47	
Maximum	234.63	1352.90	230.81	95.13	2.68	5.35	3.29	22.62	119.23	

SD standard deviation, nq not quantified

Fig. 2 PCA of physicochemical characteristics and minerals in honey samples: projection of physicochemical characteristics and minerals (variables: pH, moisture, ash, electrical conductivity, brix, free acidity, lactonic acidity, total acidity, HMF, diastase and invertase activities, Ca, K, Na, Mg, P; CB1, CB2, CB3, CB4, CB5, CB6, CB7, CB8, CB9, CB10, CB11, CB12, CB13, CB14, CB1 and CB16) into the plane composed by the principal axes F1 and F2 (73.55%)

Conclusion

Castelo Branco presents a high floral diversity which is reflected on the complexity of the honey's chemical composition. All of the samples analysed presented high quality levels, with good freshness, maturity and proper processing. However, we can highlight among them the CB2 sample, which presented the highest levels of minerals, high values of pH, moisture, ash, electrical conductivity and enzyme activity (diastase and invertase) associated with low levels of sugar and acidity. This is a very promising sample and further studies should be performed

to evaluate the presence of other bioactive compounds and its impact on human health.

Acknowledgements This work is supported by FEDER funds through the POCI—COMPETE 2020—Operational Programme Competitiveness and Internationalisation in Axis I—Strengthening research, technological development and innovation (Project POCI-01-0145-FEDER-007491) and National Funds by FCT—Foundation for Science and Technology (Project UID/Multi/00709/2013). Luís R. Silva (SFRH/BPD/105263/2014) was supported by *post doc* grants from FCT.

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