DOI: 10.1515/JAS-2016-0002 J. APIC. SCI. VOL. 60 NO. 1 2016

DE GRUYTER OPEN

Original Article

CHARACTERISATION AND ANTIOXIDANT CAPACITY OF SWEET CHESTNUT HONEY PRODUCED IN NORTH-WEST SPAIN

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Received 11 February 2015; accepted 28 January 2016

Abstract

In recent years, authentic foodstuffs have became a major requirement for consumers and producers worldwide. Honey has increased in popularity since it is associated with a natural diet, and because of honey's authentic origin. The present study investigated the palynological characteristics, physicochemical parameters, total phenol content, flavonoid content, and radical scavenging activity of 41 sweet chestnut (*Castanea sativa*) honeys from the northwestern part of Spain. These honeys were characterised by high values of electrical conductivity, pH, diastase content, and colour. All the samples showed a pollen combination of Castanea sativa-Rubus-Cytisus type-Erica. Fructose and glucose were 37.2% and 25.9%, while other sugars were less than 5%. Regarding the mineral content K, was the main with a mean value of 260.2 mg/100g. Other elements as Mg with a mean value of 17.1 mg/100g, Ca (mean value of 15.8 mg/100g) and P (mean value of 12.8 mg/100g) were well represented in this honey type. The phenol and flavonoid content were high (mean values of 129.8 mg/100g and 9.0 mg/100g, respectively). Multivariate statistical techniques showed the close relationship of colour, Mg, P, phenols, melezitose, and flavonoids, and the radical scavenging activity.

Keywords: authenticity, chemical components, DPPH, phenolic content, Spain, sweet chestnut honey

INTRODUCTION

As a guarantee of quality, the EU requires that the botanical origin and the geographical origin as well as the physicochemical characteristics of honey, are noted. There is no specific legislation for quality control of the different honey types so there is no safety assurance for consumers that demand more information (Juan-Borrás et al., 2015). Some honey types have higher values on the market because of the special sensorial properties of the honeys or for the major content of compounds with antioxidant and antimicrobial activity (Bertoncelj et al., 2007; Küçük et al., 2007; Escuredo et al., 2013a; Perna et al., 2013). Therefore, the characterisation of the different honey types, and information about their properties, increase the commercial value of this product. In Spain, several honey types are produced, but detailed investigations on the physicochemical and biological properties are limited (Escuredo et al., 2013b; Castro-Vázquez et al., 2014).

On the other hand, the use of honey as a therapeutic substance has been revalorised in a more scientific setting. Studies have demonstrated the antibacterial, anti-inflammatory, and antitumoral properties of honey (Bertoncelj et al., 2007; Kücük et al., 2007; Sarikaya et al., 2009). Diverse phytochemicals substances biosynthesised by plants have an antioxidant activity. This means that plants may be used as a natural source of free radical-scavenging compounds (Pichichero, Canuti, & Canini, 2009). Honeybees collect nectar from plants; consequently, bioactive components can be transferred to honey. The direct relationship between the phenolic content and antioxidant activity in different honey types has been cited (Bertoncelj et al., 2007; Lachman et al., 2010; Escuredo et al., 2013b; Perna et al., 2013). The presence of phenolic compounds makes honey, along with fruits

and vegetables, a nutritional source of natural antioxidants responsible for protecting human health. This is the reason for the increasing commercial interest in the determination of the antioxidant capacity of the unifloral honeys (Atanassova & Tonkov, 2013). The antioxidant activity of phenolic compounds are related to a number of different mechanisms, such as free radical-scavenging, hydrogen-donation, quenching the singlet oxygen, metal ion chelation, and acting as a substrate for radicals such as superoxide and hydroxyl (Küçük et al., 2007). Thus, in recent years, the evaluation of the antioxidant capacity of biological samples has been developed using several methods. However, in honey samples the most often used method to measure the free radical-scavenging activity (reflects total antioxidant capacity) is the DPPH method (Ferreira et al., 2009; Escuredo et al., 2013a).

Chestnut forests are one of the main habitats for honey production in the mountainous areas of the Iberian Peninsula. The northwestern region constitutes more than 70% of the total chestnut area (Melicharová & Vizoso-Arribe, 2012). These are areas characterised by the presence of chestnut trees, mainly *Castanea sativa* (sweet chestnut) and to a lesser extent, other deciduous trees such as *Quercus*. In recent decades, there has been an increase in the chestnut trees by culture and natural regeneration because of changes in land use and the abandonment of agriculture, especially in areas having an Atlantic climate. The importance of chestnut for honey production is due to the plant's abundant nectar secretion being one of the best nectar and pollen producers in Europe (Persano-Oddo & Piro, 2004).

The purpose of this work was to characterise the chestnut honeys produced in mountainous areas of the northwest part of Spain. The characterisation includes the physicochemical properties, palynological analysis, mineral components, and the phenol and flavonoid content. Evaluation of the radical-scavenging activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was also done.

MATERIAL AND METHODS

Honey samples

The present work was carried out on 41 sweet chestnut honeys collected for four consecutive years directly from beekeepers. The botanical origin of the samples was confirmed by palynological analysis.

Palynological analysis

The pollen analysis was performed according to the method established by Louveaux, Maurizio, & Vorwoh (1978). Ten grams of honey were dissolved in bidistilled water and centrifuged at 4500 rpm (3373 g) for 10 min. The obtained sediment was redissolved and centrifuged for other 5 min. The final volume of the sediment was used to prepare the slide. The pollen spectra were determined by counting and identifying a minimum of 800 pollen grains using a Nikon Optiphot II microscope (400x and 1000x, when needed). The results were expressed as the percentage of the pollen type over the pollen spectra of the sample.

Physicochemical analysis

The moisture of the honey was determined using the refractive index at 20ºC and was measured with a refractometer (ABBE URA-2WAJ-325; Auxilab S.L., Navarra, Spain). The results were expressed as the percentage of moisture. The measurements of pH and electrical conductivity (EC) were performed directly on a solution of honey (5 g) dissolved in bidistilled water (25 ml) with a pH meter (Crison micropH 2001; Crison Instruments S.A., Barcelona, Spain) and a portable conductivity meter (Knick Portamess® 913 Conductivity, Beuckestr, Berlin). For the latter, the results were expressed as mS/cm.

The hydroxymethylfurfural (HMF) content in honey was determined using the White spectrophotometric method. This method takes into account the difference between the UV absorbance at 284 nm of a honey solution (0.2 g/ml) and the same solution after the addition of bisulphite. The hydroxymethylfurfural content was calculated after subtraction of the background absorbance at 336 nm (Jenway

6305 UV-Visible Spectrophotometer, UK). The results were expressed in mg/kg.

The diastase activity was evaluated based on the hydrolysis of starch by the enzyme diastase present in a honey buffer solution at 40°C. The result of the hydrolysis yielded a blue colour. The absorbance was determined spectrophotometrically at 660 nm with a UV-VIS spectrophotometer (Jenway 6305 UV-Visible Spectrophotometer, UK) until there was an endpoint of less than 0.235. Diastase activity referred to as ID in the Schade number, corresponds with Gothe number, or g starch hydrolysed h^{-1} at 40°C per 100 g honey.

Colour was measured with a digital Hanna instrument (HANNA C 221 Honey Colour Analyzer, Rhode Island, USA). The results were expressed using the Pfund scale in mm.

Radical scavenging activity, and the phenol and flavonoid content analysis

The scavenging activity of honey samples for the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured as described by Brand-Williams, Culivier, & Berset, (1995). The amount of 0.3 ml of a honey solution dissolved in methanol (0.1 g/ml) was mixed with 2.7 ml of a DPPH solution (6×10^{-5} M). The sample mixture and a DPPH solution (blank) were kept in the dark at room temperature for 30 min. Then, the absorbance was measured at 517 nm with a UV–vis spectrophotometer (Jenway 6305, UK). The antioxidant activity of each sample was calculated as the percentage of RSA (radical scavenging activity) using the formula: RSA = $[(A_B - A_A)/A_B] \times 100$, where A_B is the absorbance of the DPPH solution and A_A is the absorbance of the honey sample solution.

The Folin-Ciocalteu method was used to determine the total phenolic content as reported by Singleton & Rossi (1965). The Folin-Ciocalteu reagent oxidised the phenolic compounds inducing a dark blue colour. As a first step, a calibration curve was obtained by mixing the Folin-Ciocalteu reagent with variously concentrated gallic acid solutions (0.01 to 0.50 mg/ml). The linearity was 0.997 (R^2). Solutions of honey samples (0.1 g/ml) and Folin-Ciocalteu reagent were prepared. The absorbance of solutions was measured at 765 nm by a UV–vis spectrophotometer (Jenway 6305, UK). The phenol content was expressed as the mg gallic acid equivalent per 100 g of honey.

The total flavonoid content was determined using the Dowd method as adapted by Arvouet-Grand et al., (1994), using quercetin as the reference. The method uses a solution of aluminium chloride that reacts with the flavonoids present in a honey sample's solution (0.33 g/ml). Solutions developed a yellow colour. Absorbance of the solutions was determined spectrophotometrically at 425 nm. The total flavonoid content was determined using a calibration curve with different quercetin volumes (0.002 to 0.01 mg/ml) whose linearity was 0.998 (R^2). Finally, the results were expressed as the mg equivalent of quercetin per 100 g of honey.

Mineral composition analysis

The mineral composition of honey was quantified using an Atomic Absorption Spectrophotometer (Varian SpectraAA-600; Agilent Technologies, Santa Clara, CA, USA). Aliquots of 0.5 g of honey properly homogenised, were transferred into teflon-coated vessels and digested in a microwave oven (CEM MARSX press model) after adding 5 ml of 9:2 HNO_3-H_2O_2 (Caroli et al., 1999). The computed minerals were K, Ca, Fe, Mg, Na, P, Zn, and Cu.

Sugar composition analysis

The quantification of the sugars was performed using an ion Dionex ICS-3000 chromatography system (Sunnyvale, California, EEUU). The separation of sugars was performed with a Carbo Pac PA1 column (3 X 250 mm). A pulsed amperometric detector (PAD) with a gradient of two mobile phases (A and B), was used to detect the sugars. Phase A involved ultrapure water, while phase B involved 200 mM NaOH (HPLC grade, Merck). The sugar content of the honey solutions (10 mg/l) was calculated using the calibration curves of the standard solution for each pure sugar (Sigma, Aldrich). The identified sugars were fructose, glucose,

sucrose, melezitose, trehalose, and maltose. The acquisition of all the chromatograms was performed with CHROMELEON Chromatography Management System.

Statistical analysis

In order to evaluate the statistical relationships between the physicochemical variables and antioxidant components of honey, Principal Components Analysis, Spearman's correlations, and linear regression analysis were applied. The significance was calculated for $P<0.05$. A multiple linear regression analysis was applied using the physicochemical parameters and antioxidant activity as the dependent variable. These statistical analyses were carried out with the SPSS Statistic 19.0 and XLSTAT 7.5.2 software for Windows.

RESULTS

Melissopalynological profile

The palynological analysis let us determine the pollen spectra expressed as the relative percentages of the different identified pollen grains in the sample. A total of 71 different pollen types were identified. The most frequent pollen types and their botanical families were summarised in Table 1. The most frequent botanical families were Fagaceae, Rosaceae, Ericaceae, and Leguminosae. *Castanea* pollen was present with a range between 70.4% and 90.2% of the pollen spectra. Relatively high percentages of *Rubus* in the samples were also found, with maximum values of 23.6%. Other important pollen types were *Cytisus, Erica*, and *Eucalyptus.* Other less frequent pollen types were found in the honey samples; *Quercus, Trifolium* type, *Plantago, Crataegus monogyna* type, Poaceae, *Campanula*

Table 1
Representation of the main pollen types present in chestnut honeys and their frequency class.

Family	Pollen type	Rep. (%)	Range (%)	Frequency class
Fagaceae	*Castanea sativa*	100.0	70.4-90.2	D
Rosaceae	*Rubus*	100.0	1.8-23.6	A
Leguminosae	*Cytisus* type	100.0	0.1-5.3	I
Ericaceae	*Erica*	100.0	0.7-13.7	I
Fagaceae	*Quercus*	73.2	0.0-1.1	R
Myrtaceae	*Eucalyptus*	56.1	0.0-4.0	I
Leguminosae	*Trifolium* type	51.2	0.0-1.3	R
Plantaginaceae	*Plantago*	53.7	0.0-1.0	R
Rosaceae	*Crataegus monogyna* type	51.2	0.0 1.9	R
Poaceae	Poaceae	46.3	0.0-0.7	R
Campanulaceae	*Campanula* type	41.5	0.0-1.5	R
Rhamnaceae	*Frangula alnus*	36.6	0.0-0.8	R
Salicaceae	*Salix*	36.6	0.0-0.7	R
Resedaceae	*Sesamoides*	34.1	0.0-0.5	R
Scrophulariaceae	*Scrophularia* type	34.1	0.0-0.3	R
Rosaceae	*Prunus* type	31.7	0.0-1.3	R
Boraginaceae	*Echium*	31.7	0.0-0.7	R
Cistaceae	*Cistus psilosepalus*	29.3	0.0-0.5	R
Umbelliferae	*Conium maculatum* type	29.3	0.0-0.4	R
Compositae	*Taraxacum officinale*	26.8	0.0-0.4	R
Cruciferae	*Brassica* type	22.0	0.0-0.5	R

Rep. (%), percentage of the samples in which the pollen type was present; D, dominant pollen (>45%); A, accompanying pollen (15-45%); I, important pollen (3-15%); R, minor (<3%).

type*, Frangula alnus, Salix, Sesamoides, Scrophularia* type, and *Prunus* type.
These samples were rich in pollen with an average pollen content of 29800 pollen grains per g of honey. Seven samples had a pollen content higher than 50000 pollen grains per gram and four had below 10000 pollen grains per gram of honey.

Physicochemical characteristics
The physicochemical characteristics of the chestnut honey samples are summarised in Table 2. The mean value of humidity was 17.9%, the mean for electrical conductivity was 1.1 mS/cm and for pH was 4.6. The studied samples were dark or dark amber in colour, with a mean value of 138 mm Pfund. Other parameters, such as HMF content and diastase activity, showed that samples were fresh honeys, with mean values of 1.7 mg/kg and 22.1 ID, respectively.
The average concentration of the identified mineral elements of the chestnut honeys was 310.0 mg/100g. The major mineral was K, with a mean value of 260.2 mg/100g. Chestnut honey was also rich in Ca and Mg followed by P, Na, Fe,

Table 2

Descriptive analysis of physicochemical parameters, phenol and flavonoid content, antioxidant activity, minerals and sugars.

	Mean ± SD	Limit of confidence 95%		Minimum	Maximum
		Min.	Max.		
Moisture (%)	17.9 ± 0.9	17.6	18.2	15.8	19.8
EC (mS/cm)	1.1 ± 0.2	1.0	1.1	0.7	1.5
pH	4.6 ± 0.4	4.5	4.7	3.9	5.2
Colour (mm Pfund)	138 ± 16.5	133	144	100	150
HMF (mg/kg)	1.7 ± 2.7	0.8	2.5	0.0	13.9
Diastase (ID)	22.1 ± 6.7	19.9	24.2	9.7	36.7
Phenols (mg/100g)	129.8 ± 26.5	121.5	138.2	73.0	190.3
Flavonoids (mg/100g)	9.0 ± 1.9	8.4	9.6	4.4	12.4
RSA (%)	66.8 ± 12.9	62.7	70.9	32.2	81.3
K (mg/100g)	260.2 ± 55.4	242.7	277.7	161.5	377.0
Ca (mg/100g)	15.8 ± 6.8	13.7	18.0	6.8	47.6
Fe (mg/100g)	0.3 ± 0.2	0.2	0.4	0.0	0.7
Mg (mg/100g)	17.1 ± 9.6	14.1	20.1	3.0	40.2
Na (mg/100g)	3.4 ± 1.8	2.9	4.0	1.1	8.4
P (mg/100g)	12.8 ± 6.5	10.8	14.9	4.8	31.5
Zn (mg/100g)	0.2 ± 0.1	0.1	0.2	0.0	0.7
Cu (mg/100g)	0.2 ± 0.1	0.1	0.2	0.1	0.7
∑minerals (mg/100g)	310.0 ± 65.8	289.2	330.7	191.1	438.8
Fructose (%)	37.2 ± 2.6	36.4	38.1	33.2	45.0
Glucose (%)	25.9 ± 2.7	25.0	26.7	20.1	30.5
Maltose (%)	1.1 ± 0.8	0.8	1.3	0.4	4.0
Sucrose (%)	0.2 ± 0.3	0.1	0.3	0.0	1.6
Trehalose (%)	0.1 ± 0.1	0.0	0.1	0.0	0.5
Melezitose (%)	0.1 ± 0.2	0.1	0.2	0.0	1.1
∑sugars (%)	67.1 ± 5.0	65.6	68.7	59.8	80.3

SD: standard deviation; EC: electrical conductivity.

Zn, and Cu. Fructose was the main carbohydrate in all the samples, with a mean value of 37.2%, followed by glucose with an average of 25.9% (Table 2). Other sugars were maltose (1.1%), sucrose (0.2 %), trehalose (0.1%), and melezitose (0.1%).

Radical scavenging activity, and the total phenol content and flavonoid content

The results showed that sweet chestnut honey from the northwestern part of Spain exhibited a high antioxidant activity with an average of 66.8% (Table 2). The mean phenol content was 129.8 mg/100g, with a range between 73.0 mg/100g and 190.3 mg/100g while the mean value for the flavonoid content was 9.0 mg/100g, with a range between 4.4mg/100g and 12.4 mg/100g.

Relationship between antioxidant activity and physicochemical components

Results showed significantly positive correlations between some physicochemical parameters as pH, electrical conductivity, colour, flavonoids, mineral content, and RSA (Table 4). There is a strong relationship between the botanical origin, polyphenolic compounds, and antioxidant activity of honey (Bertoncelj et al., 2007; Lachman et al., 2010; Escuredo et al., 2013a,b, 2015). In this sense, the dark honeys such as chestnut honey have a higher polyphenol content, and consequently, a higher antioxidant capacity (Atrouse, Oran, & Al-Abbadi, 2004). These correlations reinforce the fact that chestnut honeys have a high pH, high electrical conductivity, dark colour, high flavonoid and mineral content, and high antioxidant capacity. Furthermore, melezitose had a significantly positive correlation with electrical conductivity, colour, and RSA.

Fifteen parameters were used to calculate the Principal Component Analysis (Table 3 and Fig. 1): four main minerals (K, Mg, P, Ca), RSA, colour, electrical conductivity, flavonoids, pH, phenols, melezitose, diastase content,

Table 3

Components extracted and weights of each variable performed by PCA.

Component number	1	2	3	4	5
Eigenvalue	5.1	2.5	1.6	1.3	1.0
Variance (%)	34.1	16.8	10.9	9.0	6.8
Variance cumulative (%)	34.1	50.8	61.8	70.8	77.5
Component score coefficients					
RSA	0.366	0.100	-0.147	-0.050	-0.277
Mg	0.357	0.165	-0.326	-0.061	0.102
P	0.337	0.089	-0.280	0.144	0.239
Colour	0.336	0.196	0.112	-0.013	-0.110
EC	0.314	-0.072	0.049	-0.484	-0.157
K	0.313	-0.339	0.212	-0.025	0.115
Flavonoids	0.267	0.353	0.269	0.105	-0.373
pH	0.259	-0.317	-0.006	0.189	0.377
Ca	0.247	-0.160	-0.084	0.072	-0.121
Phenols	0.178	0.250	0.454	0.376	-0.035
Melezitose	0.174	0.133	0.112	-0.438	0.530
Castanea sativa pollen	0.102	-0.030	0.454	0.117	0.291
Diastase	0.026	-0.369	0.227	-0.450	-0.320
Glucose	-0.085	0.517	-0.174	-0.270	0.113
Fructose	-0.188	0.248	0.385	-0.253	0.166

EC: electrical conductivity.

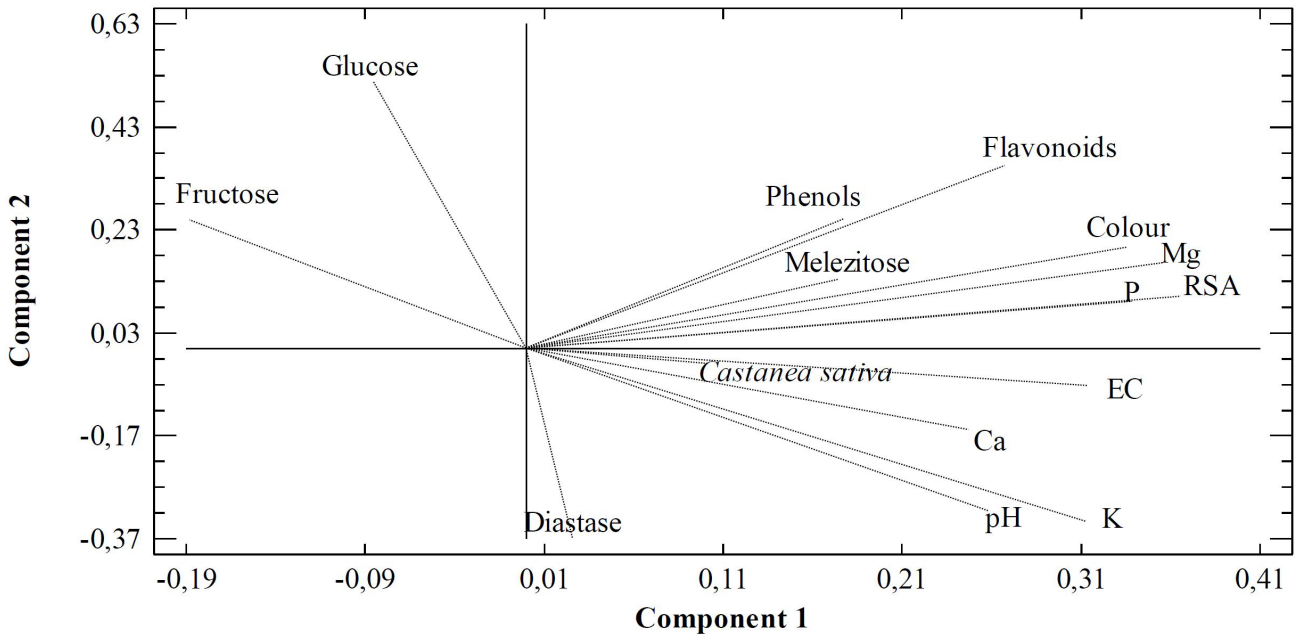

Fig. 1. PCA analysis of physicochemical parameters and antioxidant compounds in chestnut honey. Projection into the plane composed by the principal components. Ec: electrical conductivity

glucose, fructose, and *Castanea sativa* pollen. This statistical method is a common technique used to find patterns in data showing similarities between samples and identifying which variables determine these similarities and in what way. The variables were grouped onto five principal components that explained 77.5% of the total data variance (Table 3). The first component explained the 34.0% and the two first components explained the 50.8% of the total variance. The vectors representing the main physicochemical parameters were highly correlated (Fig. 1). Some variables pH, K, Ca, electrical conductivity, *Castanea sativa* pollen, P, Mg, melezitose, colour, phenols, and flavonoids, were situated near and on the right of the plot, while fructose and glucose were on the left.

The direct relationship of the radical scavenging activity (RSA) of chestnut honey with the combination of various physicochemical parameters was evaluated by a multiple lineal regression analysis. This analysis allowed us to evaluate the variables that interfere the most in the antioxidant activity of this type of honey. The results showed a linear relationship for the total antioxidant capacity with flavonoids and Mg. The adjusted model were represented by a R^2 value of 0.6 ($F = 31.7$; $P < 0.001$).

DISCUSSION

The pollen spectra of the honeys reflected the Atlantic vegetation of the northwestern part of Spain. *Castanea* produces a good quantity of nectar and pollen during their flowering period (between May in the lowest lands, and July in the mountains), and *Castanea* is the main resource for honeybees in the area (Seijo, Escuredo, & Fernández-González, 2011; Escuredo et al., 2014). This taxon appears frequently with values near 80% of pollen spectra (70-90%) in these honeys. Some authors considered *Castanea* pollen strongly over-represented. Many laboratories require a percentage of at least 90%, with more than 100000 PG/10 g of honey, before accepting the honey as unifloral (Persano-Oddo & Piro, 2004). Although the studied chestnut honeys contained a medium-high pollen content (only four samples had a pollen content below 100000 PG/10 g honey), the percentage of *Castanea* pollen in the pollen spectra was relatively low. The presence of the pollen combination *Castanea-Rubus-Cytisus-Erica* in all the pollen spectra of the honeys is characteristic for chestnut honeys from this geographical origin.

Table 4

Spearman's rank correlation analysis among physicochemical parameters and the dominant pollen.

	Glucose	Fructose	Sucrose	Melezitose	Maltose	EC	pH	Colour	Phenols	Flavonoids	RSA	*Castanea sativa*
K	-0.533**	-0.302	0.349*	0.294	0.280	0.601**	0.734**	0.350*	0.329*	0.148	0.399**	0.332*
Ca	-0.115	-0.334*	0.351*	0.401**	0.133	0.615**	0.418**	0.570**	0.226	0.348*	0.495**	0.269
Fe	-0.520**	-0.248	-0.153	-0.034	0.678**	-0.163	0.669**	0.236	0.351*	-0.002	0.075	0.130
Mg	0.234	-0.459**	0.597**	0.552**	-0.146	0.636**	0.316*	0.568**	0.170	0.484**	0.715**	0.041
Na	-0.227	0.463**	-0.613**	-0.546**	0.303	-0.563**	-0.213	-0.240	0.127	-0.125	-0.445**	0.037
P	0.009	-0.447**	0.465**	0.391*	0.125	0.537**	0.512**	0.554**	0.337*	0.438**	0.719**	0.082
Zn	-0.452**	-0.232	-0.281	-0.375*	0.705**	-0.315*	0.351*	-0.061	0.299	-0.135	-0.080	0.164
Cu	-0.265	-0.081	-0.257	-0.265	0.672**	-0.421**	0.378*	0.029	0.249	-0.127	-0.192	0.027
Glucose	1.000	0.366*	0.055	0.205	-0.316*	0.095	-0.439**	0.200	-0.107	0.202	-0.101	-0.164
Fructose		1.000	-0.217	-0.098	-0.074	-0.103	-0.384*	-0.207	0.018	-0.103	-0.458**	-0.030
Sucrose			1.000	0.506**	-0.283	0.528**	0.177	0.256	-0.044	0.117	0.407**	0.210
Melezitose				1.000	-0.214	0.549**	0.230	0.415**	-0.059	0.163	0.368*	0.041
Maltose					1.000	-0.243	0.502**	0.052	0.153	-0.229	-0.130	0.023
EC						1.000	0.279	0.493**	0.128	0.378*	0.551**	0.110
pH							1.000	0.348*	0.181	-0.046	0.242	0.133
Colour								1.000	0.548**	0.731**	0.586**	0.165
Phenols									1.000	0.732**	0.233	0.318*
Flavonoids										1.000	0.573**	0.231

EC: electrical conductivity; *P< 0.05; **P<0.001

Regarding the physicochemical analysis, the high electrical conductivity (minimum of 0.7 mS/cm), high colour (minimum of 100 mm pfund), pH (minimum of 3.9), the F/G ratio (from 1.2 to 1.9), and the values of G/W ratio (from 1.0 to 1.8), performs a typical physico-chemical pattern common to chestnut honeys from other countries (Persano-Oddo & Piro, 2004; Bertoncelj et al., 2007; Küçük et al., 2007; Kolayli et al., 2008; Giorgi et al., 2011; Atanassova & Tonkov, 2013). Some parameters, such as the diastase content, had a high standard deviation between samples. The diastase activity of honey, is closely related with the botanical origin but the abundance of the nectar secretion and how quickly is it collected and transferred to the comb by the honeybee affect the final content. Also, aging and heat treatment reduce diastase content. More than 50% of the samples had values of diastase activity higher than 15.

Despite there being a larger number of papers regarding the metal content in honey few papers refer to the botanical origin (Bobis et al., 2008; Nozal-Nalda et al., 2005; Bogdanov et al., 2007; Escuredo et al., 2015). In general, light blossom honeys have a lower mineral content than dark honeys such as honeydew, chestnut or heather. In this sense, the mineral content in chestnut honey, especially of K, P, Mg, and Ca, can be considered higher than in other blossom honeys (Küçük et al., 2007; Kropf et al., 2010; Escuredo et al., 2013a; Bilandžić et al., 2014). The presence of these elements in honey is highly dependent on the geographical origin since soil composition differs. The kinetics of the mineral sorption and desorption capacity of soils is a major factor determining the dynamics of the elements in the soil-plant system and their availability. It is of note that the content of some minerals like K, Mg, Ca, and P had a high standard deviation and probably

the origin of the soil, from granite or slate is the reason of this high variation.

The sugars in honey are responsible for many of a honey's physicochemical properties such as viscosity, hygroscopicity, and crystallisation. Sugars represent the main components of honey. The sugar component depends mostly on the botanical and geographical origins, and on lesser factors, such as the weather, processing, and storage conditions (Ouchemoukh et al., 2010; Dobre et al., 2012). Higher fructose and glucose contents were found in other unifloral honeys, such as acacia, rape, sunflower or lime (Bentabol et al., 2011; Was et al., 2011; Escuredo et al., 2014). The presence of low percentages of reduced sugars and a high amount of melezitose has been related to the presence of honeydew in honeys (Sanz et al., 2005, Vela, De Lorenzo, & Perez, 2007; Ruoff et al., 2007, Rybak-Chmielewska et al., 2013). Nevertheless, only three samples had a value of fructose and glucose less below 59%, and only one had a melezitose content higher than 1% (1.1%).

In the last decade the antioxidant activity of foods, including honey, was studied to find out the effects of the antioxidant activity on animal health. One of the methods widely used is the DPPH scavenging ability (Ahn et al., 2007). The high antioxidant potential for sweet chestnut honeys was cited by other authors, for honeys from Turkey (Küçük et al., 2007; Kolayli et al., 2008; Sarikaya et al., 2009), Italy (Pichichero, Canuti, & Canini, 2009; Giorgi et al., 2011; Perna et al., 2013), and Slovenia (Bertoncelj et al., 2007). Sarikaya et al., (2009) noted that chestnut propolis and chestnut honeys may protect humans from deleterious oxidative processes as a result of the antioxidative activity of the chestnut propolis and chestnut honeys. This honey type could be included with the unifloral honeys produced in Spain that have the highest antioxidant activity. The relationship between the botanical origin, the total content of phenols and flavonoids, and antioxidant activity of honeys from Galicia was analysed in detail by Escuredo et al., (2013a). They concluded that chestnut honeys are the unifloral honeys with the highest antioxidant capacity when compared with other types of honey such as eucalyptus or blackberry honey. Finally the high phenol content was in concordance with other dark honeys from other geographical areas (Küçük et al., 2007; Kolayli et al., 2008; Sarikaya et al., 2009; Giorgi et al., 2011; Perna et al., 2013). The importance of the phenolic compounds on the honey matrix was considered by various researches because phenolic compounds are important components for human health (Bertoncelj et al., 2007; Küçük et al., 2007). Of the phenolic compounds, some flavonoids have been studied in depth for their health benefits. Honey since it is rich in flavonoids was considered a natural food source of flavonoids (Alvarez-Suarez et al., 2010). The phenolic and flavonoids content of the studied samples had a high standard variation as was also mentioned for the mineral content. Some particularities of the samples, like geographical origin, could explain these differences since a strong relationship between antioxidant capacity, flavonoids content, and Mg, was found.

The present work showed that Castanea sativa honeys from the northwestern part of Spain were characterised by high values of electrical conductivity, pH, F/G ratio, and colour. Information regarding the palynological characterisation of this honey type reveals a pollen combination of Castanea sativa-Rubus-Cytisus type-Erica, present in all the samples. At the same time, these honeys can be considered rich in the minor components of phenols, flavonoids, and minerals. Although these are minor components in honey, they provide chemical information related to the botanical origin of honey and contribute to the characterisation of the honey. The evaluation of the radical scavenging activity of honey revealed its high antioxidant capacity compared to other unifloral honeys. In fact, the high correlation coefficient between antioxidant activity, some minerals (Mg and P), and flavonoids, indicated that these compounds are important for the antioxidant behaviour of this honey type.

ACKNOWLEDGMENTS

The authors wish to thank the collaboration of the beekeepers and the Protected Geographical Indication "Miel de Galicia" who have generously provided the samples. The results of mineral content and sugar content were obtained in collaboration with CACTI research service of the University of Vigo (Spain).

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