INFLUENCE OF THE BOTANICAL ORIGIN OF HONEY FROM NORTH WESTERN SPAIN IN SOME ANTIOXIDANT COMPONENTS

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Received 26 October 2011; accepted 13 February 2013

S u m m a r y

Honeys were studied in order to know the relationships between their botanical origin, the total content of phenols and flavonoids, and the 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The principal floral resources for honeybees in the studied area were *Castanea sativa*, *Rubus*, *Eucalyptus, Erica*, and *Cytisus.*

Results showed the influence of *Erica* and *Castanea* in the phenol content, flavonoids content, and the antioxidant activity of honeys. The presence of *Erica* in honeys was related to the highest phenol content; so heather honeys had a mean content of 178.9 mg/100g. Honeys with high *Castanea* pollen content had the highest value of flavonoids (7.7 mg/100g) and a minor value of half maximal inhibitory concentration (IC_{50}) (9.4 mg/ml). Eucalyptus and blackberry were the honeys with minor contents of these compounds. A stepwise linear regression was used to estimate the phenol content of honeys. The contribution of *Erica* and *Castanea* was highlighted in the obtained model ($R^2 = 0.61$), with significant p-value (< 0.01).

Keywords: honey, *Erica*, *Castanea*, phenol content, flavonoid content, DPPH, Spain.

INTRODUCTION

Honey is well known in traditional medicine. Since ancient times honey has been used for its therapeutic and nutritive qualities (Ball, 2007). It is a natural combination that contains a complex mixture of sugars, minerals, proteins, vitamins, enzymes, organic acids, polyphenols and other phytochemicals mainly from vegetal origin.

A great number of medicinal and aromatic herbs as well as the fruits and leaves of some berry plants biosynthesize the phytochemicals possessing antioxidant activities that are used as a natural sources of free radical scavenging compounds. Some of these plants are used by bees to produce honey. Consequently, plant origin bioactive components are transferred to honey (Lachman et al., 2010).

Many authors have demonstrated that honey serves as a source of natural antioxidants (Chen et al., 2000; Nagai et al., 2006), especially polyphenols and flavonoids, which are effective in reducing the risk of heart disease, cancer, inflammatory processes, asthma, infected wounds, chronic wounds, skin ulcers, and cataracts (Yao et al., 2004). The composition and antioxidant capacity of honey depend on various factors, principally the plant resource used by the honeybees.

The aim of this work was to study the relationship among the botanical origin of the honey and the presence of

some antioxidant components such as polyphenols, flavonoids, and the radical scavenging activity.

MATERIAL AND METHODS

Honey samples

In this study, we have analyzed 174 honeys produced in northwest Spain during 2008, 2009 and 2010. The samples were collected directly from beekeepers and stored in the Laboratory of Plant Biology and Soil Sciences of the Faculty of Sciences of Ourense for further analysis.

Melissopalynological analysis

The study of the pollen content in honey was carried out in accordance to the melissopalynological method (Louveaux et al., 1978). Honey (10 g) was dissolved in bi-distilled water and centrifuged at 4500 rpm (3373 g) for 10 minutes. The obtained sediment was then re-dissolved and centrifuged for 5 minutes. Pollen spectra were performed counting a minimum of 800 pollen grains in two 100 µl aliquots using light microscopy (400X or 1000X, when necessary). The results were expressed as percentages.

Colour

The colour was measured with a HANNA Honey Colour C221 colorimeter (Woonsocket, Rhode Island, USA). The samples were previously homogenized and the crystallized samples were heated to 45 ºC until complete clarification. The results were expressed using the Pfund scale.

Phenol content

The total phenol content was determined using the Folin-Ciocalteu method (Singleton and Rossi, 1965). Each honey sample was diluted with bi-distilled water (0.1 g/ml). Ten ml of bi-distilled water and 1 ml of Folin-Ciocalteu reagent were added to 1 ml of the diluted honey sample. The mixture was gently agitated and left to rest for 2 min. Then, 4 ml of disodium tricarbonate (Na_2CO_3) and bi-distilled water for a final volume of 25 ml, were added to the mixture. The solution was incubated for 1 h at room temperature, in the dark. Finally, the absorbance was read at 765 nm. Gallic acid was used as reference for the calibration curve (Prior et al., 2005). A stock solution of gallic acid was prepared for the final dilutions (0.0008 mg/ml - 0.016 mg/ml). The linearity was 0.998 (R^2). The results were expressed as mg gallic acid/100 g of honey.

Flavonoid content

The total flavonoid content was measured using the Dowd method as adapted by Arvouet-Grand et al., (1994). Each honey sample was diluted in bi-distilled water (0.3 g/ml). The honey solution (2 millilitres) was mixed with 0.5 ml of aluminium chloride ($AlCl_3$) and bi-distilled water was added for a final volume of 25 ml. The solution was kept in the dark for 30 min., and the absorbance was measured at 425 nm. The quercetin was used as a reference, and different concentrations were used for calibration (0.002 mg/ml - 0.01 mg/ml). The linearity was 0.998 (R^2). The results were expressed as mg quercetin/100g of honey.

2.2 - diphenyl -1- picrylhydrazyl (DPPH) radical scavenging

The DPPH radical scavenging assay is based on the measurement of the antioxidant scavenging ability of 2.2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors.

Honey samples were dissolved in methanol (0.1 g/ml). The honey solution (0.3 ml) was mixed with 2.7 ml of a DPPH solution (6 x 10^{-5} mol/l). The resulting solution had a purple colour, which decreased in intensity according to the presence of antioxidant compounds. The sample solution and a DPPH solution (blank) were maintained in the dark at room temperature for 30 min. (Okada and Okada, 1998) before the absorbance was measured at 520 nm (Ferreira et al., 2009).

The DPPH inhibition (%) was calculated with this formula: % $DPPH_{inh.} = [(A_B - A_A) / A_B] \times 100$, where A_B is the absorption of a blank

sample; and A_A is the absorption of the honey sample. The concentration of the honey required to scavenge 50% of DPPH (IC_{50}) was calculated using a standard curve of ascorbic acid (0.0006 - 0.1 mg/ml). The IC_{50} (mg/ml) was calculated by a linear regression analysis and was an indicator of the concentration of honey providing 50% of radical scavenging activity.

Oxidation index

The oxidation index was determined with a honey solution in ethanol (0.04 g/ml). The filtered honey solution (2 ml) was placed in an assay tube. In the same tube 1 ml of a (20%) sulphuric acid (SO_4H_2) solution and a drop of 0.1N potassium permanganate ($KMnO_4$) were added (Bedascarrasbure et al., 2006). The time (in seconds) necessary for the solution to become clear was measured.

Statistical analysis

All the assays on the honey samples were performed in duplicate. Data were expressed as mean ± standard deviation of duplicate measures. Differences between the samples were analyzed by Bonferroni and ANOVA tests. Spearman's rank correlation analysis was applied between the phenolic content, flavonoid content, $IC_{50,}$ and the principal pollen types identified in the honey samples studied. Also, stepwise regression analysis was performed using the obtained variables to analyze which of them influenced the phenol content of these honeys. Cluster analysis was realized to group the samples according the studied variables. The statistical analyses were performed with the SPSS Statistic 17.0 software for Windows.

RESULTS AND DISCUSSION

Melissopalynological analysis

Pollen analysis is an important tool to identify the floral resources used by the bees to produce the honey. Four types of monofloral honeys (chestnut, eucalyptus, heather, and blackberry), and polyfloral honey, were studied. In these samples, 96 pollen types corresponding to 50 botanical families were identified. Nevertheless, only a few plants were important to honey production in the area. The best represented families were Fagaceae, Leguminosae, Ericaceae, Rosaceae, and Myrtaceae. The principal pollen types in each type of honey are given in Table 1.

Castanea sativa was a very frequent pollen type in the samples studied. It was present in all the polyfloral honey, blackberry honey and, of course, in chestnut honey. The same was with *Rubus* pollen which was present in all polyfloral honey, heather honey, and blackberry honey. Both taxa produce a high quantity of nectar and pollen during their flowering (between May in the lowest lands, and July in the mountains), and are very important resources for honeys in the territory (Seijo and Jato, 1998; Seijo et al., 2011). The mentioned pollen types reached a high percentage in the pollen spectra of the honey (maximum of 90.2% for *C. sativa* and maximum of 91.3% for *Rubus*).

Erica pollen (principally from *E. umbellata*, *E. arborea*, *E. australis*, and *E. cinerea*) and *Cytisus* pollen type (obtained from Leguminosae shrubs such as *Cytisus multiflorus*, *Cytisus striatus*, *Cytisus scoparius*, and *Genista florida*) were identified in all heather honeys, eucalyptus honey, and polyfloral honeys. *Erica* pollen had a maximum of 49.4% in the pollen spectra of honeys, whereas *Cytisus* pollen had a maximum of 46.2%. *Erica* plants and *Cytisus* type plants dominated the Atlantic shrub communities in Galicia and occupied around 630,000 ha, so they were one of the most abundant resources for honeybees in the area.

Other important plant for honeybees is *Eucalyptus*. In the coastal lands, this taxon provides a good quantity of nectar and pollen in the early spring. The maximum value in the pollen spectra of honey was 94.8%. Also, this pollen type is present in all polyfloral honeys and heather honeys.

Other pollen types, such as *Echium*, *Brassica*, *Trifolium*, *Campanula*, *Crataegus monogyna*, *Salix*, *Frangula alnus*, and *Conium maculatum* were of a minor importance in the samples (values

Table 1.

Principal pollen types identified in honey

		Chestnut honey (n = 35)					Blackberry honey (n = 40)					Heather honey (n = 11)					Eucalyptus honey (n = 16)					Polyfloral honey (n = 72)				
Family	**Pollen type**	%	R	I	A	D	%	R	I	A	D	%	R	I	A	D	%	R	I	A	D	%	R	I	A	D
Boraginaceae	*Echium*	46	15	1	-	-	78	23	8	-	-	82	9	-	-	-	75	12	-	-	-	76	46	9	-	-
Campanulaceae	*Campanula* t.	34	12	-	-	-	50	19	1	-	-	27	3	-	-	-	13	2	-	-	-	25	18	-	-	-
Cistaceae	*Cistus psilosepalus*	23	8	-	-	-	50	20	-	-	-	27	3	-	-	-	6	1	-	-	-	33	24	-	-	-
Cruciferae	*Brassica* t.	29	10	-	-	-	30	12	-	-	-	64	7	-	-	-	81	13	-	-	-	54	37	2	-	-
Ericaceae	*Calluna vulgaris*	17	6	-	-	-	3	1	-	-	-	73	8	-	-	-	6	1	-	-	-	14	10	-	-	-
Ericaceae	*Erica*	97	17	17	-	-	95	24	12	2	-	100	-	-	10	1	100	10	6	-	-	100	20	37	15	-
Fagaceae	*Quercus*	57	20	-	-	-	68	25	1	1	-	64	6	1	-	-	94	12	3	-	-	68	47	2	-	-
Fagaceae	*Castanea sativa*	100	-	-	-	35	100	4	6	28	2	91	2	1	6	1	88	7	6	1	-	100	3	3	32	34
Leguminosae	*Trifolium* t.	57	19	1	-	-	93	31	6	-	-	82	9	-	-	-	69	11	-	-	-	85	52	9	-	-
Leguminosae	*Lotus* t.	20	7	-	-	-	18	7	-	-	-	27	2	1	-	-	31	5	-	-	-	19	14	-	-	-
Leguminosae	*Cytisus* t.	97	23	11	-	-	98	19	20	-	-	100	4	3	4	-	100	11	4	1	-	100	28	37	6	1
Myrtaceae	*Eucalyptus*	71	20	5	-	-	63	20	5	-	-	100	6	3	2	-	100	-	-	-	16	96	26	19	18	6
Rhamnaceae	*Frangula alnus*	31	11	-	-	-	48	17	2	-	-	27	2	1	-	-	19	3	-	-	-	35	25	-	-	-
Rosaceae	*Prunus* t.	31	11	-	-	-	43	17	-	-	-	27	3	-	-	-	69	11	-	-	-	44	32	-	-	-
Rosaceae	*Crataegus* t.	46	16	-	-	-	45	18	-	-	-	55	6	-	-	-	63	10	-	-	-	43	30	1	-	-
Rosaceae	*Rubus*	97	1	24	9	-	100	-	-	-	40	100	4	5	2	-	94	12	3	-	-	100	6	18	48	-
Salicaceae	Salix	34	12	-	-	-	45	18	-	-	-	73	8	-	-	-	100	15	1	-	-	76	47	6	2	-
Scrophulariaceae	*Scrophularia* t.	37	13	-	-	-	28	11	-	-	-	64	7	-	-	-	69	11	-	-	-	42	30	-	-	-
Umbelliferae	*Conium maculatum* t.	23	7	1	-	-	35	14	-	-	-	55	6	-	-	-	81	12	1	-	-	46	32	1	-	-

t.: pollen grain with similar morphology for various genera.
%: percentage representation, R: minor pollen (<3% of pollen spectra), I: important pollen (3-15% of pollen spectra), A: secondary pollen (15-45% of pollen spectra), D: dominant pollen (>45% of pollen spectra).

Table 2.

Phenol content, flavonoid content, oxidation index, IC_{50} and colour of the samples studied according to honey type; values are expressed as mean ± standard deviation.

	Chestnut honey	Blackberry honey	Heather honey	Eucalyptus honey	Polyfloral honey
Phenol content (mg/100 g)	131.3 ± 28.7 bcd	89.3 ± 30.6 ace	178.9 ± 59.8 abde	76.8 ± 42.2 ace	112.1 ± 37.0 bcd
Flavonoid content (mg/100 g)	7.7 ± 2.5 bde	5.8 ± 2.2 a	6.5 ± 2.0	4.2 ± 1.3 a	5.5 ± 1.8 a
Oxidation Index (s)	7.8 ± 6.2 d	11.3 ± 7.4	11.9 ± 7.0	14.8 ± 4.8 a	11.7 ± 7.3
IC_{50} (mg/ml)	9.4 ± 6.1 de	11.5 ± 7.7 d	10.8 ± 6.3	18.4 ± 8.0 abe	14.6 ± 8.0 ad
Colour (pfund)	124 ± 26 bde	95 ± 29 a	120 ± 32 bd	79 ± 21 ac	98 ± 24 a

The letters shows the means with significant differences determined by Bonferroni analysis ($P<0.05$); a=chestnut honey, b=blackberry honey, c=heather honey, d=eucalyptus honey, e=polyfloral honey; IC_{50} - half inhibitory concentration.

less than 10%). The pollen analysis indicated that honeybees used wild flowers to produce honeys. Pollens from cultivated plants were rarely represented.

Chestnut honey was characterized by the presence of *C. sativa* pollen (more than 70%), *Rubus* as accompanying pollen (15 - 45%) and *Cytisus* type, *Erica*, *Eucalyptus*, *Echium* or *Conium maculatum* type as important pollen (3 - 15%). Other identified pollens were of minor importance. Blackberry honey was also characterized by the presence of *Rubus* pollen in more than 45% of the pollen spectra of honeys always accompanied by *C. sativa*. Important pollens were *Cytisus* type, *Erica*, *Trifolium* type, *Echium*, and *Quercus* among others. Heather honey presented *Erica* pollen in more than 26% of pollen spectra, being an underrepresented pollen type. As accompanying pollen, *C. sativa*, *Cytisus*, *Eucalyptus*, and *Rubus* were identified. Finally, eucalyptus honey presented more than 70% of *Eucalyptus* pollen. The secondary pollen types in this last honey were *C. sativa*, *Cytisus*, *Rubus*, and *Quercus*.

Colour

The honeys ranged from an amber colour to a dark colour (from 150 mm to 39 mm on the Pfund scale). Eucalyptus samples and blackberry samples were the lighter honeys with an amber colour. The rest had darker colours. High electrical conductivity, dark colour, and high mineral content are related to the presence of honeydew in honey (Vela et al., 2007). However, some other honeys from *C. sativa*, *Erica, Calluna* presented the same darker colour; frequently more than 100 mm Pfund (Von der Ohe et al., 2004; Waś et al., 2011).

Polyphenols, flavonoids and DPPH-IC_{50}

Eucalyptus honeys and blackberry honeys had the lowest values of phenolic content, with a mean of 76.8 mg/100 g and 89.3 mg/100 g, respectively (Tab. 2). The highest values were obtained for chestnut honeys with a mean content of 131.3 mg/100 g, and heather honeys with a mean content of 178.9 mg/100 g. While the polyfloral honeys had a mean phenol content of 112.1 mg/100 g.

Related to flavonoids content, the unifloral honeys had smaller differences in the mean content. The chestnut honeys and heather honeys had the highest values, with a mean content of 7.7 mg/100 g and 6.5 mg/100 g, respectively. The blackberry honeys and polyfloral honeys had values close to 6 mg/100 g, and the eucalyptus honeys had the lowest content with a mean value of 4.2 mg/100 g. As shown in Table 2, there were significant differences among the honeys with respect to the phenolic content. Heather honeys were different from all others types of honeys ($P<0.05$), while blackberry honeys were similar to eucalyptus honeys ($P<0.05$). The group of chestnut honeys did not show significant differences when compared with polyfloral honeys. *C. sativa* had an important presence in polyfloral honeys, therefore, the phenol

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Table 3.

Summary of the stepwise regression analysis for phenol content

Model summary					
R	R^2	R^2 adjusted	Standard Error	F	P
0.78	0.61	0.61	27.31	89.53	0.00
Coefficients					
	B	Standard Error B	Beta	t	P
Intercepted	23.9	7.7		3.1	0.00
Erica	2.6	0.2	0.6	10.6	0.00
Colour	0.5	0.08	0.3	6.4	0.00
C. sativa	0.4	0.1	0.2	4.2	0.00
Phenol content = 2.6 Erica + 0.5 Colour + 0.4 C. sativa + 23.9					

contents were similar. Concerning flavonoid content, chestnut honeys showed significant differences compared to eucalyptus honeys, blackberry honeys, and polyfloral honeys ($P<0.05$).

The mean phenolic content of the studied samples was similar to the results reported by Vit et al. (2008) for Czech honeys. *Trigona carbonaria* honeys had inferior values of polyphenol contents and higher values of flavonoid contents (Persano-Oddo et al., 2008). On the other hand, a higher polyphenol content for honeydew honeys have been reported by some authors (Al et al., 2009; Pichichero et al., 2009; Soria et al., 2005). Acacia honey from Burkina Faso had similar phenol and flavonoid contents to those of the blackberry honeys included in this study (Meda et al., 2005), however, acacia honey from Romania had a lower content (Al et al., 2009).

The oxidation index varied between a mean time of 7.8 s for chestnut honeys and 14.8 s for eucalyptus honeys (Tab. 2). These unifloral honeys showed high significant differences ($P<0.05$). The rest of the studied unifloral honeys and the polyfloral honeys took about 12 s to become clear.

The DPPH method was used for determination of the antioxidant activity the first time by Brand-Williams et al. (1995). The DPPH assay is considered a rapid and easy assay to evaluate the scavenging activity of antioxidants because the radical compound is stable and does not have to be generated as in other radical scavenging assays (Sánchez-Moreno, 2002; Tabart et al., 2009; Karadag et al., 2009). Different researchers have used different initial radical concentrations and reaction times (Sánchez-Moreno, 1998).

The results were expressed as the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50% (IC_{50}). Table 2 shows that eucalyptus honeys and polyfloral honeys were the least active, with a mean IC_{50} value of 18.4 mg/ml and 14.6 mg/ml, respectively. Chestnut honeys had IC_{50} (9.4 mg/ml) making them the most active honey. These honey types showed significant differences when compared eucalyptus honeys and polyfloral honeys ($P<0.05$). Eucalyptus honeys also showed significant differences when compared with blackberry honeys ($P<0.05$). The results obtained were in close agreement with the IC_{50} values reported by Bertoncelj et al. (2007) for chestnut honeys from Slovenian. Minor values of IC_{50} were reported for other chestnut honeys and strawberry tree honeys (Beretta et al., 2005).

Statistical analysis

Different statistical analyses were performed to study the influence of the botanical origin of honeys in the phenol and flavonoid contents and the radical scavenging activity. Spearman linear correlation analysis showed that the phenol contents and flavonoid contents were strongly correlated ($P<0.01$) with positive

Table 4.

Centroids of each class obtained by the cluster analysis

Classes	*Erica* (%)	*C. sativa* (%)	*Cytisus* T. (%)	*Eucalyptus* (%)	*Rubus* (%)	Phenol content (mg/100g)	Flavonoid content (mg/100g)
1	11.1 bc	54.4 bc	5.7	5.6 b	16.9 bc	145.0 bc	7.0 bc
2 (A)	4.5 a	7.2 ac	6.0	67.9 c	4.0ac	81.0 a	4.6 a
2 (B)	4.1 a	35.6 ab	4.4	5.1 b	43.5ab	83.4 a	5.1 a

The letters shows the significant differences determined by Bonferroni analysis ($P<0.05$).
a: class 1, b: class 2(A), c: class 2(B).

value. The oxidation index and the IC_{50} showed negative correlation coefficients with phenol and flavonoid contents ($P<0.01$) whereas colour was positively correlated with them ($P<0.01$) and negatively correlated with oxidation index and IC_{50}. Other researchers (Bertoncelj et al., 2007; Beretta et al., 2005; Frankel et al., 1998) also found a relationship between the antioxidant activity and the colour of the honey.

In relation to the botanical origin of honeys, the statistical analysis indicated a positive correlation between the phenol content and the following taxa: *C. sativa* ($P<0.01$), and *Erica* ($P<0.01$). In contrast, blackberry and eucalyptus had a negative correlation with the phenol content ($P<0.01$). The best positive correlation coefficient of flavonoids was with *Erica* and *C. sativa* ($P<0.01$) and there was a negative correlation with *Eucalyptus* pollen ($P<0.01$). Then again *Erica* ($P<0.01$) and *C. sativa* ($P<0.05$) had negative correlation coefficients with IC_{50}, whereas *Eucalyptus* had a positive correlation coefficient ($P<0.01$). The oxidation index values also represent the effect of *C. sativa* ($P<0.05$) *Erica* ($P<0.05$), and *Eucalyptus* ($P<0.01$) in the antioxidant capacity of honey.

Stepwise regression analysis ($R^2 = 0.61$ and $F = 89.5$; $P<0.01$) is shown in Table 3. The multiple linear regression model indicated that the polyphenol content was strongly related with both the botanical origin of honeys and the colour. The best result obtained was with *Erica* and *Castanea* pollen content. The relation between the colour, the phenol content, and botanical origin of the studied honey are in accordance with other results (Vela et al., 2007; Estevinho et al., 2008) which confirmed that the highest polyphenolic content was in dark honeys. The relation of *Castanea* and *Erica* with phenol and flavonoid contents has been mentioned by other authors (Küçük et al., 2007; Nazemiyeh et al., 2008; Pavlović et al,. 2009). The antioxidant capacity of the plants of the Ericaceae family has also been mentioned (Luís et al., 2009; Ay et al., 2007) and their influence in honeys from the north of Portugal was pointed out by Estevinho et al. (2008).

A cluster analysis was performed with the most representative variables (Tab. 4). Ward agglomeration method was used for grouping the samples. The analysis differentiated two classes (48.7% samples in class 1 and 51.3% samples in class 2). In class 1, there were mainly the honeys with a high content of *Erica* pollen (11.1%) and *C. sativa* pollen (54.4%), and high phenol content (145.0 mg/100g) and flavonoid content (7.0 mg/100g). Again Class 2 was divided into two groups (A, B). Samples in group A had a high *Eucalyptus* pollen content (mean of 67.9%), low *Erica* pollen (4.5%) and *C. sativa* pollen (7.2%), low phenol content (81 mg/100g) and low flavonoid content (4.6 mg/100g). Group B included samples with high *Rubus* pollen content (43.5%); low phenol content (83.4 mg/100g) and low flavonoid content (5.1 mg/100g). Significant differences ($P<0.05$) between the three groups established by cluster analysis were obtained by the variance analysis (ANOVA). These results allowed differentiating heather honey and chestnut honey as the highest phenol and flavonoid

content, and eucalyptus honeys and blackberry honey as the lowest.

CONCLUSION

The botanical origin of honeys heavily influenced the phenol content and antioxidant activity of honeys. *Erica* pollen and *C. sativa* pollen were associated with the highest phenolic content and flavonoid content of the studied honeys. Honey with a high presence of *Eucalyptus* and *Rubus* pollen had the lowest content. Therefore, heather and chestnut honeys had the highest amount of these compounds and the best antioxidant activity indicated as IC_{50}.

ACKNOWLEDGMENTS

The authors especially want to thank the participation of beekeepers of PGI Mel de Galicia. This research project was funded by FEADER 2008-5, with the economical support of Conselleria de Medio Rural (Xunta de Galicia), Ministerio de Medio Ambiente, Medio Rural y Marino and FEADER.

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WPŁYW POCHODZENIA BOTANICZNEGO MIODÓW Z PÓŁNOCNO-ZACHODNIEJ HISZPANII NA ZAWARTOŚĆ NIEKTÓRYCH PRZECIWUTLENIACZY

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S t r e s z c z e n i e

Celem badań było znalezienie związku pomiędzy całkowitą zawartością fenoli i flawonoidów w miodzie oraz jego zdolnością dezaktywacji rodnika DPPH a jego pochodzeniem botanicznym. W badaniach uwzględniono cztery odmiany miodu (kasztanowy, eukaliptusowy, wrzosowy i jeżynowy) oraz miód wielokwiatowy. W miodach tych stwierdzono obecność pyłku pochodzącego z 96 gatunków roślin należących do 50 rodzin. Nie mniej jednak dla produkcji miodu na tym terenie znaczenie miało jedynie kilka gatunków roślin. W badanych miodach w największych ilościach występował pyłek: *Castanea sativa*, *Rubus*, *Eucalyptus* oraz gatunków rodzaju *Erica*. Inne rodzaje pyłku miały niewielkie znaczenie: *Echium*, *Brassica*, *Trifolium*, *Campanulla*, *Crateagus monogyna*, *Salix*, *Frangula alnus*, *Conium maculatum*.

Barwa badanych miodów wahała się od bursztynowej do ciemnej (od 150 mm do 39 mm w skali Pfunda). Miody eukaliptusowy i jeżynowy były jaśniejsze, miały bursztynowy kolor. Te odmiany miodu charakteryzowały się najniższą zawartością związków fenylowych. Najwyższą zawartość fenoli oznaczono z kolei w miodach wrzosowym i kasztanowym. Średnia zawartość związków fenolowych w tych odmianach wyniosła odpowiednio 178,9 i 131,3 mg/100g miodu. Taka sama zależność widoczna była również dla zawartości flawonoidów. Najwięcej flawonoidów zawierały miody kasztanowy i wrzosowy, odpowiednio 7,7 i 6,5 mg/100g. Biorąc pod uwagę zdolność dezaktywacji rodników, miód kasztanowy miał niską wartość IC_{50} (9,4 mg/ml) i niewielką wartość wskaźnika utleniania (7,8 s), będąc najaktywniejszą odmianą miodu. Miód eukaliptusowy był najmniej aktywny.

Analiza współczynnika korelacji liniowej Spearmana wykazała, że zawartości fenoli i flawonoidów były ze sobą silnie pozytywnie skorelowane. Natomiast wskaźnik utleniania i wartość IC_{50} były negatywnie skorelowane z zawartością fenoli i flawonoidów. Barwa miodu była pozytywnie skorelowany z zawartością fenoli i flawonoidów, a negatywnie ze wskaźnikiem utleniania i wartością IC_{50}. Analiza wielokrotnej regresji liniowej wykazała, że zawartość polifenoli była silnie związana z pochodzeniem botanicznym miodu i jego barwą. Najlepsze wyniki otrzymano dla zawartości pyłku *Erica* i *Castanea*.

Słowa kluczowe: miód, *Erica, Castanea*, zawartość fenoli, zawartość flawonoidów, DPPH, Hiszpania.