HONEYS FROM NW SPAIN: BOTANICAL ORIGIN RELATED TO PHENOLICS CONTENT

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INTRODUCTION

In the year 2007 the PGI Miel de Galicia has been recognized in the EU (DOCE L 192/11 of July 24 2007). Actually there are more than 31000 beehives protected by the denomination and situated in the whole Galician. The PGI honey production in the 2008 was approximately 600.000 kg.

Honey is a natural food to which are attributed many biological properties. The presence of antioxidant compounds in the composition of honey confers it a good part of these properties. Several of these compounds are phenols and flavonoids. These are also related with the color of the product and proceed from nectar and pollen. In 2008, the group of Beekeeping of the University of Vigo and the Council Regulator of PGI "Miel de Galicia" has begun a study of the nutritious and biological properties of the honey. Until the moment 74 samples of honey produced in Galicia (NW Spain) during 2008, have been studied. In this work we present the results of the determination of botanical origin, color, total phenolics content, flavonoids content and oxidation index.

MATERIAL AND METHODS

In this work we study different parameters in honeys produced in North West Spain during 2008. Phenol content it has been determined by a method based in Maldonado *et al.*, 2006 using as a reference Gallic acid. 2g of honey were diluted in 20 mL of water. We take 1 mL of this solution and add 10 mL of water and 1 mL of the Folin-Ciocalteu reagent. Next 4 mL of Na_2CO_3 was added. Final volume of 25 mL was kept in darkness. Absorbance was measured at 765 nm. Flavonoids content were determined using as reference pattern quercetina. 10 g of honey were diluted in 30 mL of water. Absorbance was read at 425 nm. The antioxidant activity has been determined by means of a DPPH radical-scavenging activity (Ferreira *et al.*, 2009). Absorbance was measured at 520 nm. The ascorbic acid has been used as reference to determine the DPPH decoloration and this is expressed as percentage of radical-scavenging activity (RSA). For the determination of the oxidation index we were diluted 2 g of honey in 50 mL of ethanol. The seconds takes in the decoloration a drop of $KMnO_4$ were counted. PH was measured directly on honey dissolved in water. Colour was made by colorimetry and the results are expressed using the Pfund scale. Electrical conductivity was determined with a conductivity meter. The last determinations were carried out immediately after receipt the samples. The methodology for pollen analysis was a modified method based in Louveaux *et al.* (1978). Pollen spectra was performed counting a minimum of 800 pollen grains, cuantitative analysis was made with a volumetric method counting an alicuot of 10µL.

RESULTS AND DISCUSSION

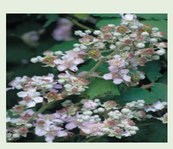
Descriptive analysis

	Descriptive analysis (Chesnut honey)					
	Mean	St. Dv.	Confidence -95%	Confidence 95%	Min	Max
Mean phenolic (mg/100g)	110,93	29,22	86,49	135,36	70,70	155,68
Mean flavonoid (mg/100g)	4,98	1,94	3,36	6,60	3,41	9,00
RSA (%)	36	24	15	56	19	91
Oxidation index (s)	15	10	7	23	4	27
N. pollen grain/g	33417	31604	6996	59839	2070	90232
pH	4,18	0,38	3,86	4,49	3,72	4,91
Colour (pfund)	99	26	77	121	68	150
CE (mS/cm)	0,748	0,231	0,555	0,941	0,443	1,108

The chestnut is the most representative tree for the production of honey inside Galicia. It produces monofloral honey with amber dark colour, wood scent of medium persistence, lightly bitter taste and astringency. The plants that usually accompany to the chestnut tree in the production of honey are brambles and heaths. It is the type of honey with the highest phenolic content.

	Descriptive analysis (Multifloral)					
	Mean	St. Dv.	Confidence -95%	Confidence 95%	Min	Max
Mean phenolic (mg/100g)	96,41	31,27	86,79	106,04	39,69	157,30
Mean flavonoid (mg/100g)	4,65	1,39	4,22	5,08	1,28	8,27
RSA (%)	35	14	31	39	9	76
Oxidation index (s)	14	8	11	17	4	46
N. pollen grain/g	26051	22790	19037	33065	1132	101105
pH	4,10	0,32	4,00	4,20	3,29	4,74
Colour (pfund)	95	24	88	103	42	150
CE (mS/cm)	0,649	0,219	0,581	0,716	0,285	1,208

The main botanical origin of any one honeys can to be any one of those mentioned previously. Is very frequent the presence of heaths that tinge the sensorial characteristics of our honeys. These honeys present a wide variation range in their phenol content.



	Descriptive analysis (Blackberry honey)					
	Mean	St. Dv.	Confidence -95%	Confidence 95%	Min	Max
Mean phenolic (mg/100g)	75,48	24,33	64,70	86,27	33,91	109,77
Mean flavonoid (mg/100g)	4,81	1,92	3,96	5,66	1,40	7,86
RSA (%)	48	24	37	59	14	92
Oxidation index (s)	14	9	10	18	4	29
N. pollen grain/g	47343	37481	30725	63961	4582	130832
pH	4,30	0,38	4,13	4,46	3,33	4,94
Colour (pfund)	92	33	78	107	39	150
CE (mS/cm)	0,656	0,251	0,545	0,767	0,224	1,067

The bramble is very important for honey production in our territory. This genus presents a very constant flowering with good nectar secretion even under adverse meteorological conditions. It produces monoflorals honeys of amber to dark amber colour, floral/frutal scent and taste clearly sweet. The phenolic content is lower than chestnut tree honeys.

	Descriptive analysis (*Eucalyptus* honey)
	Mean
Mean phenolic (mg/100g)	74,46
Mean flavonoid (mg/100g)	4,84
RSA (%)	32
Oxidation index (s)	9
N. pollen grain/g	35700
pH	4,05
Colour (pfund)	75
CE (mS/cm)	0,659

Eucalyptus honeys produce in the coast of Galicia and they come from *E. globulus*. They are honeys with amber colour, waxy scent and lightly sour taste. It is a type of honey of very unstable production, existing years of excellent production and years of null production. This last was the case of the year 2008. The phenol content is similar to *Rubus* honey.

Correlation analysis

In the tables is indicated the result of the correlation analysis among the phenol and flavonoids content and other studied parameters.

Regarding botanical origin it is of highlighting that several pollen types provide significant coefficients. The *Erica* genus, *Campanula*, *Cytisus, Castanea*, *Eucalyptus* or *Rubus* are the most representative in honeys. Other pollen types have low frequency or occasional presence, in this case it is necessary other studies for confirmation the results. *Erica* sp. has a great relation with flavonoids and total phenol content.

	Mean phenolic (mg/100g)	
	Spearman coefficient	p
Mean Flavonoid (mg/100g)	0,390***	0,001
RSA (%)	0,249**	0,032
Oxidation index (s)	-0,200*	0,087
N. pollen grain/g	-0,306***	0,008
Colour (pfund)	0,656***	0,000
Erica arborea (%)	0,429***	0,000
Erica australis (%)	0,261**	0,025
Erica sp. (%)	0,710***	0,000
Erica umbellata (%)	0,617***	0,000
T. *Erica cinerea* (%)	0,476***	0,000
Castanea sativa (%)	0,217*	0,063
T. *Cytisus* (%)	0,411***	0,000
T. *Lotus* (%)	0,234**	0,045
Plantago (%)	0,348***	0,002
Rubus (%)	-0,341***	0,003
***p<0,01; **p<0,05; *p<0,1		

	Mean flavonoid (mg/100g)	
	Spearman coefficient	p
Mean phenolic (mg/100g)	0,390***	0,001
RSA (%)	0,792***	0,000
Oxidation index (s)	-0,708***	0,000
Colour (pfund)	0,745***	0,000
CE (mS/cm)	0,585***	0,000
T. *Campanula* (%)	0,372***	0,001
Centaurea (%)	-0,242**	0,038
Erica arborea (%)	0,444***	0,000
Erica sp. (%)	0,334***	0,004
Erica umbellata (%)	0,443***	0,000
Castanea sativa (%)	-0,191*	0,102
T. *Cytisus* (%)	0,437***	0,000
T. *Trifolium* (%)	-0,526***	0,000
Eucalyptus (%)	-0,369***	0,001
Frangula alnus (%)	0,367***	0,001
***p<0,01; **p<0,05; *p<0,1		

Regression analysis

A great part of our territory is occupied mainly for Ericaceae and Fabaceae (*Ulex*, *Cytisus*, *Genista* or *Adenocarpus* principally). *Erica* sp. (*E. australis*, *E. arborea*, *E. umbellata* and *E. Cinerea*, the most abundant species) have a good nectar pattern and are very important for honey production. These are plants very attractive for the bees but frequently beekeepers do not obtained monoflorals honeys.

This genus seems to have a great relationship with phenol and flavonoids content in the honey. Tables show regression models for flavonoids and phenol content. In the case of flavonoids the regression explain 79,4% of the cases.

In the case of RSA (%) the relation obtained with the content in flavonoids explains 63% of the cases.

Model summary						
Phenolic	R	R^2	R^2 ajusted	Est. Error	F	p
	0,779	0,607	0,584	19,885	26,668	0,000
Coefficients						
		B	Est. Err. B	Beta	t	p
Intercpt.		105,051	30,622		3,431	
pH		-27,155	8,179	-0,308	-3,320	0,001
Colour		0,772	0,123	0,672	6,289	0,000
Erica		0,968	0,464	0,203	2,086	0,041
Castanea		0,491	0,123	0,303	3,980	0,000

Phenolic (mg/100g) = (-27,155 * pH) + (0,772 * Colour) + (0,968 * *Erica*) + (0,491 * *Castanea*) + 105,051

Model summary						
Flavonoid	R	R^2	R^2 ajusted	Est. Error	F	p
	0,896	0,802	0,794	0,724	94,586	0,000
Coefficients						
		B	Est. Err. B	Beta	t	p
Intercpt.		-7,514	1,091		-6,885	0,000
pH		2,182	0,298	0,479	7,323	0,000
Colour		0,031	0,004	0,525	6,994	0,000
Erica		0,038	0,017	0,153	2,234	0,029

Flavonoid (mg/100g) = (2,182 * pH) + (0,031 * Colour) + (0,038 * *Erica*) – 7,514

Model summary						
RSA (%)	R	R^2	R^2 ajusted	Est. Error	F	p
	0,795	0,632	0,627	11,785	123,512	0,000
Coefficients						
		B	Est. Err. B	Beta	t	p
Intercpt.		-6,664	4,317		-1,544	0,127
Flavonoid		9,611	0,865	0,795	11,114	0,000

RSA (%) = (9,611 * Flavonoid) – 6,664

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