NITROGEN CONTENT OF HONEYS FROM NW SPAIN

ESCUREDO O., MIGUEZ M., SEIJO M. C. & CHOUZA M. (*)

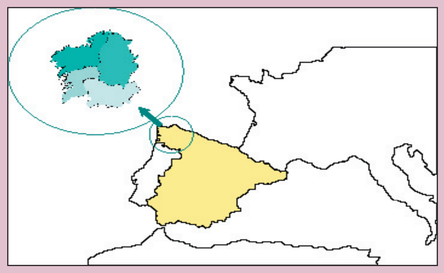
(*) Faculty of Sciences. University of Vigo. Campus As Lagoas, 32004 Ourense. Spain.

e-mail: mcoello@uvigo.es. Tel: +34 988387057. Fax: +34 988387001

INTRODUCTION

Honey is a natural food, traditionally used as an alimentary supplement without the addition of any substance during its elaboration. In our territory honey production is principally based in medium or small apiaries (less than 30 hives), beekeepers collected one harvest per year mainly in later summer (August-September).

Principal honeybee resources are trees: *Eucalyptus globulus* in the coast and *Castanea sativa* inland. However, different species of *Rubus* are essential for honey production in this territory. Their flowering occurs during May and June, nectar secretion are good and constant, so that bees collect and important quantity of nectar for them. The last three taxa and *Erica* sp. are the most important taxa in honeys from Galicia.



Honey have a low protein content with various essentials amino acids so that, it is considered an important nutrient. Pollen is the main source of honey nitrogen content and the origin of amino acids in honey is attributed to both animal and vegetal sources (Rebane and Herodes, 2008). According to Louveaux (1968) the proteins are responsible for the thixotropy of honey of *Calluna* (Ericaceae).

The presence of proteins causes a lower surface tension in honey, this fact produces a marked tendency to foam and form scum and encourages formation of fine air bubbles (White and Doner, 1980).

MATERIAL AND METHODS

We analyzed 74 honeys from North west Spain. Honeys are from *Rubus*, *Castanea sativa*, *Erica* and *Eucalyptus* principally.

It has been determined nitrogen content, protein content, pH, colour, electrical conductivity, pollen spectra and pollen content. All the analyses were carried out in duplicate.

The nitrogen content was determined by the Kjeldahl method (AOAC XVIII edition, 2005). 1g of dissecated honey with 15 mL of SO_4H_2 is digested at least during four hours. After this, it was added NaOH and the resulting solution was distilled to a final volumen of 150 mL. Finally the distilled solution was titrate with HCl. The protein content is calculated with a conversion factor of 6,25.

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 was 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.

With the subject of studying the association between the nitrogen content in honey with different physicochemical parameters such as pH, color, electrical conductivity, pollen grains per gram of honey and botanical origin, we realized a statistical analysis with SPSS Statistics 17.0.

RESULTS AND DISCUSSION

In this work we have identified 31 unifloral honeys (Blackberry, Chestnut and Eucalyptus) and 43 multifloral honeys.

In general nitrogen content in honey is low, varied from 0,05% to 0,22% with a mean of 0,12±0,03 %. The different honey types have a similar content. The same occurs with protein content, in both cases, multifloral honeys have the minimum and the maximum values (0,315% and 1,367% respectively).

PH varied from 3,29 to 4,94 with a mean of 4,16±0,35, having blackberry honey the maximum values.

The colour and electrical conductivity in this honey samples were variable. The a range of colour varied from 39 mm to 150 mm with a mean value of 95±27 mm. The mean value of electrical conductivity was 0,662±0,227 mS/cm with a minimum value to 0,224 mS/cm and maximun value to 1,208 mS/cm.

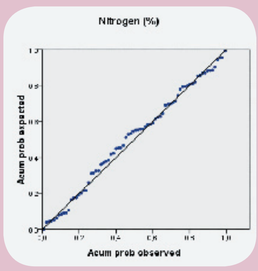
The most frequent pollen types are *Castanea sativa*, *Rubus*, *Erica* and *Cytisus*. *Castanea sativa* and *Cytisus* are present in all the honeys.

	Nitrogen (%)	
	Spearman coefficient	p
N. pollen grain/g	0,269*	0,021
pH	0,471**	0,000
Colour	0,505**	0,000
CE	0,619**	0,000
Echium	-0,241*	0,038
Cistus psilosepalus	0,252*	0,030
Erica arborea	0,256*	0,028
Erica sp.	0,243*	0,037
Erica umbellata	0,360**	0,002
T. *Cytisus*	0,391**	0,001
T. *Trifolium*	-0,493**	0,000
Eucalyptus	-0,257*	0,027
Plantago	-0,246*	0,035
Frangula alnus	0,280*	0,016

*p< 0,05 ;** p< 0,01

The table in the left shows the parameters statistically correlated with nitrogen content. There are positive correlations with nitrogen content (99% significance level) betwwen pH, colour, electrical conductivity, *Erica umbellata* and *Cytisus* type. With a significance level of 95% are corelated pollen grains per gram of honey and *Cistus psilosepalus*, *Erica arborea*, *Erica* sp. and *Frangula alnus.*

Taking in account the last results we made a regression analysis looking for a relation of nitrogen content and physicochemical analysis. The figure shows the acumulated probability expected in front of the acumalated probablity observed for each honey sample.



The best adjusted regression was obtained with the number of pollen grains per gram of honey, electrical conductivity and *Erica* pollen. The results of this regression analysis is significant with a F of 24,309 (p< 0,000).

Model summary						
% nitrogen	R	R^2	R^2 ajusted	Est. Error	F	p
	0,714	0,510	0,489	0,026	24,309	0,000
Coefficients						
		B	Est. Err. B	Beta	t	p
Intercpt.		0,045	0,010		4,594	0,000
N. pollen grain/g		$3,9 \times 10^{-7}$	$1,08 \times 10^{-7}$	0,320	3,614	0,001
CE		0,077	0,014	0,482	5,615	0,000
Erica		0,002	$4,9 \times 10^{-4}$	0,395	4,532	0,000

% Nitrogen = ($3,9 \times 10^{-7}$ * N. pollen grain/g) + (0,002 * *Erica*) + (0,077 * CE) + 0,045

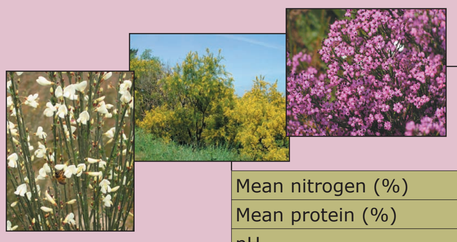
	Descriptive analysis (*Eucalyptus*)
	Mean
Mean nitrogen (%)	0,135
Mean protein (%)	0,841
pH	4,05
Colour (pfund)	75
CE (mS/cm)	0,659
N. pollen grain/g	35700
Eucalyptus (%)	75,8
Salix (%)	10,8
T. *Cytisus* (%)	6,7
Castanea sativa (%)	0,6
Rubus (%)	0,4
T. *Trifolium* (%)	0,4
Erica sp. (%)	0,3
Echium (%)	0,1
T. *Erica cinerea* (%)	0,1

The tables show the descriptive statistics for each honey type.

The chestnut honey show the highest values of conductivity and colour. The mean value of conductivity is 0,748±0,231 mS/cm and colour is 99±26 mm. Whereas blackberry honey show a mean conductivity of 0,656±0,251 and 92±33 mm. Multifloral honey are the group with high diversity in the parameters studied. pH is near 4,0 with similar standard deviation in all of the groups.

Regarding pollen analysis Chestnut honey has a minimum of 70% of *Castanea* pollen with a maximum of 87,9%. The minimum percentage of *Rubus* pollen for Blackberry honeys were 45,1% and the maximum 91,3%. Only one sample was from *Eucalyptus* with a 75,8% of their pollen. Other significative pollen types are exposed in tables. Blackberry honeys have the highest pollen content (mean of 47343 pollen grains/g honey).

To know the existence of significatives differences between honey types we also have been made an analysis of variance (ANOVA). The simbol (*) shows the level of significative differences between groups. Different letters (a= *Castanea sativa*, b= *Rubus*, c= Multifloral) show the honey groups with significative differences through Dunnett test.



	Descriptive analysis (Blackberry honey)					
	Mean	St. Dv.	Confidence -95%	Confidence 95%	Min	Max
Mean nitrogen(%)	0,122	0,036	0,106	0,138	0,067	0,202
Mean protein (%)	0,760	0,225	0,660	0,860	0,421	1,262
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
N. pollen grain/g	47343 bc*	37480	30725	63961	4581	130832
Rubus (%)	58,4 abc***	11,4	53,4	63,5	45,1	91,3
Castanea sativa (%)	26,5 abc***	12,1	21,1	31,8	1,9	52,8
Erica arborea (%)	1,9	3,1	0,5	3,3	0,0	13,3
T. *Cytisus* (%)	3,8	2,9	2,5	5,1	0,1	12,3
Eucalyptus (%)	1,3 bc***	1,9	0,4	2,2	0,0	8,8
Brassica (%)	0,1 bc**	0,1	0,0	0,1	0,0	0,5
Erica umbellata (%)	0,7 bc***	1,1	0,2	1,2	0,0	4,4
Plantago (%)	0,1 bc**	0,1	0,0	0,1	0,0	0,4
Erica sp. (%)	0,4 bc***	0,6	0,1	0,6	0,0	2,4
T. *Erica cinerea* (%)	0,2 bc**	0,3	0,0	0,3	0,0	1,2

a = *Castanea sativa* honey variable with significative differences Dunnett test (*p<0,10; **p<0,05; ***p<0,01).
b = *Rubus* honey variable with significative differences Dunnett test (*p<0,10; **p<0,05; ***p<0,01).
c = Multifloral honey variable with significative differences Dunnett test (*p<0,10; **p<0,05; ***p<0,01).

	Descriptive analysis (Multifloral honey)					
	Mean	St. Dv.	Confidence -95%	Confidence 95%	Min	Max
Mean nitrogen (%)	0,122	0,037	0,111	0,134	0,050	0,219
Mean protein (%)	0,765	0,234	0,693	0,838	0,315	1,367
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
N. pollen grain/g	26051 bc*	22790	19037	33065	1132	101105
Castanea sativa (%)	46,4 abc***	12,4	42,6	50,2	2,9	64,7
Eucalyptus (%)	8,7 bc***	12,5	4,8	12,5	0,0	58,9
T. *Cytisus* (%)	6,5 ac*	8,2	4,0	9,0	0,3	43,5
Rubus (%)	23,4 abc***	11,2	20,0	26,9	2,1	43,3
Salix (%)	1,2 ac**	3,0	0,3	2,2	0,0	16,4
Erica arborea (%)	1,4	2,9	0,5	2,3	0,0	14,8
Erica australis (%)	0,5	1,8	0,1	1,1	0,0	11,9
Erica umbellata (%)	2,4 bc***	3,1	1,4	3,4	0,0	11,6
T. *Erica cinerea* (%)	1,2 bc**	2,1	0,5	1,9	0,0	10,0
Echium (%)	1,4 ac***	2,0	0,8	2,0	0,0	9,8
T. *Trifolium* (%)	1,5	2,2	0,8	2,2	0,0	9,6
Erica sp. (%)	1,5 bc***	1,5	1,0	2,0	0,0	6,0
Quercus (%)	0,3	0,6	0,1	0,5	0,0	3,8
Plantago (%)	0,2 bc**	0,5	0,1	0,4	0,0	2,0
Brassica (%)	0,2 bc**	0,3	0,1	0,3	0,0	1,7
T. *Prunus* (%)	0,2 ac**	0,3	0,1	0,3	0,0	1,2
Cistus psilosepalus (%)	0,1 ac**	0,2	0,0	0,1	0,0	0,7

a = *Castanea sativa* honey variable with significative differences Dunnett test (*p<0,10; **p<0,05; ***p<0,01).
b = *Rubus* honey variable with significative differences Dunnett test (*p<0,10; **p<0,05; ***p<0,01).
c = Multifloral honey variable with significative differences Dunnett test (*p<0,10; **p<0,05; ***p<0,01).

	Descriptive analysis (Chestnut honey)					
	Mean	St. Dv.	Confidence -95%	Confidence 95%	Min	Max
Mean nitrogen (%)	0,118	0,038	0,086	0,149	0,067	0,168
Mean protein (%)	0,736	0,237	0,538	0,934	0,421	1,052
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
N. pollen grain/g	33417	31604	6996	59839	2070	90232
Castanea sativa (%)	75,3 abc***	5,6	70,6	80,0	70,2	87,9
Rubus (%)	9,2 abc***	5,4	4,7	13,7	0,0	15,4
Eucalyptus (%)	2,9	3,1	0,3	5,5	0,0	7,9
T. *Cytisus* (%)	3,2 ac*	2,1	1,4	4,9	0,3	5,2
Erica umbellata (%)	1,6	1,6	0,2	3,0	0,0	4,6
Erica arborea (%)	1,6	1,9	0,1	3,2	0,0	4,1
Erica sp. (%)	1,2	1,4	0,0	2,3	0,0	3,8
T. *Trifolium* (%)	0,8	0,6	0,2	1,3	0,1	1,9
T. *Erica cinerea* (%)	0,6	0,5	0,2	1,0	0,1	1,7
Erica australis (%)	0,3	0,4	0,0	0,7	0,0	1,1
Echium (%)	0,3 ac***	0,3	0,0	0,6	0,0	1,0
Quercus (%)	0,3	0,4	0,0	0,6	0,0	0,8
Salix (%)	0,1 ac**	0,1	0,0	0,2	0,0	0,3
Cistus psilosepalus (%)	0,1 ac**	0,1	0,0	0,1	0,0	0,2
T. *Prunus* (%)	0,1 ac**	0,1	0,0	0,1	0,0	0,1

a = *Castanea sativa* honey variable with significative differences Dunnett test (*p<0,10; **p<0,05; ***p<0,01).
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c = Multifloral honey variable with significative differences Dunnett test (*p<0,10; **p<0,05; ***p<0,01).

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