PHYSICO CHEMICAL CHARACTERISTICS OF PROPOLIS COLLECTED IN SANTA FE (ARGENTINE)

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ABSTRACT

Propolis is a very valuable bee product due to its antioxidant, bacteriostatic, antifungal and therapeutical properties which are related to the content of phenolic and flavonoid compounds. In this work, physico chemical characteristics and some active compounds of 42 samples collected in different phytogeographical regions of Santa Fe (Argentine) were measured. Hexane extractable substances ranged between 14.3 and 46.2%, insoluble residues between 18.0 and 22.4%; resins soluble in ethanol between 26.6 and 75.8%, phenolic compounds between 11.3 and 38.0%,loss by heating between 1.4 and 6.2% and ash between 1.8 and 2.4%. Concentrations corresponding to phenolic and flavonoid compounds obtained by HPLC show the prevalence of pinocembrin, crisine, galangine and quercetine.

The propolis differed mainly in the presence of phenolic compounds being significantly largest in pampeana area propolis samples where the plant species are in the families mirthaceas and salicaceas.

Key Words: Argentine propolis/ propolis characterization

INTRODUCTION

Raw propolis is an important product of the beehive produced by honeybees (*Apis mellifera*), gathering and transforming the bud exudates, by mixing it with waxy substances (Serra Bonhevi, 1996). It has a sweet, balsamic odour and presents variable consistency, depending on the origin and temperature. Its colour is variable, from light yellow to dark brown, with a wide range of brownish intermediate tones (Salamanca Grosso et al., 2002).

The average composition of propolis shows the presence of 20-30% waxes, resins and aromatic balsams (40-50%), essential and aromatic oils (5-10%), pollen (4-5%) and other various substances, including insoluble residue (10-30%) (Brown, 1995). Simple fractionation of propolis to obtain compounds is difficult due to its complex composition (Burdock, 1998). It can be separated into a fraction soluble in ethanol, called balsams; and into another fraction insoluble in alcohol, called waxy fraction. Balsam chemical pattern and propolis physico chemical characteristics vary according to the botanical and geographical origin (Montenegro et al., 2000).

This bee product is valuable for its antioxidant, bacteriostatic, antifungal and therapeutic properties, related to the contents of phenolic and flavonoid compounds (Salamanca Grosso et al., 2002). It is applied in medicine, cosmetics, veterinary and food industries (Maidana, 2000). Substances identified in propolis are, in general, typical constituents of food and/or food additives, known as Generally Recognized as Safe (GRAS) substances (Burdock, 1998). The main difficulty is the absence of controls on the origin and composition of the propolis produced (Maidana, 2000). Propolis standardization has not been carried out in a complete and systematic way yet.

Argentina is the third honey world producer and the second exporter. Honey production comprises also other bee-hive products such as propolis, though with a lower incidence in the apicultural activity. The main producer provinces are Buenos Aires, Entre Ríos, Córdoba and Santa Fe. The most productive areas are in the plains. There is a large amount of agricultural activity in the study region. Tree communities are very scattered and limited nowadays but it is thought they were significantly larger in the past.

The aim of this work is the physico-chemical characterization of propolis produced in the Province of Santa Fe (Argentine).

MATERIALS AND METHODS

Sampling was carried out considering agro-climatic features, latitude and flora (for honey purposes). Santa Fe (28º - 35º SL; 58º- 62º WL) was divided into its phytogeographical areas which closely correspond to honey production and bee-hive product areas. They are called *chaqueña province* in the north; *espinal province* in the centre and *pampeana province* in the south (Figure 1).

There are no woods in the *pampeana province* area and the ground is covered by grass. Its main crops are *Glicine max* (soybean) and *Zea mays* (corn). Rows of *Eucalyptus sp*. (Mirtaceae) and *Salix humboldtiana* (Argentinian willow - Salicacceae) are also present..

In the *chaqueña province*, there are *Prosopis nigra* (black algarrobo - Fabaceae), *Parkinsonia aculeata* (cina-cina - Fabaceae), *Acacia caven* (aromito - Fabaceae), *Erythrina crista-galli* (ceibo tree - Fabaceae), *Astronium Balansae* (urunday - Anacardiaceae), *Schinopsis Balansae* (red quebracho from Chaco - Anacardiaceae) and *Apidosperma quebracho-blanco* (white quebracho -

Apocianaceae). Randomly, *Sapium haematospermum* (curupí or lecherón - Euphorbiaceae) and *Sesbania punicea* (coffee plant of the coast or acacia of the swamps - Fabaceae) can be found. In the *espinal province* there are other species such as the *Phitolacca dioica* (umbra tree - Phitolacaceae), and some herbaceous such as *Stypa neesiana* (flechilla brava - Poaceae), *Sporobolus indicus* (Poaceae), *Medicago spp*. (clovers or alfalfas - Fabaceae) and *Carex bonariensis* (carex - Cyperaceae) (Quargnolo, 1982).

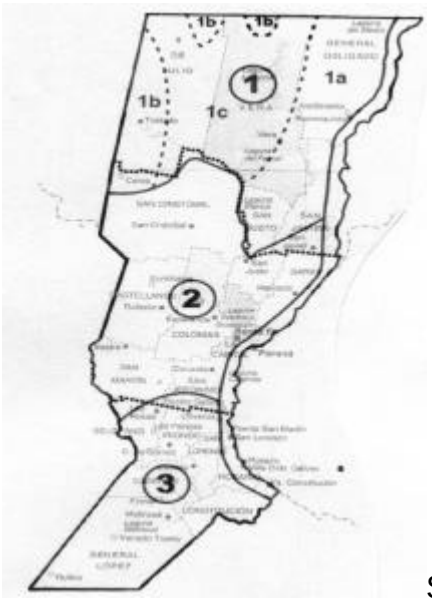

Figure 1 – Province of Santa Fe (Argentine)

1 North area – Chaqueña province
2 Center area – Espinal province
3 South area – Pampeana province

Sampling was carried out according to Standard RST-RSFSR-317-77 (1977), during 2004. Samples were collected by people from the Provincial Apicultural Programme of the Ecological Division – Ministry of Agriculture, Cattle Farming, Industry and Commerce of the Province of Santa Fe (Argentine), who employed the scraping technique following the instructions of the Apicultural Programme (PROAPI) of the National Institute of Agricultural Technology (Instituto Nacional de Tecnología Agropecuaria - INTA). A representative sample of boxes and frames of at least 25% of the bee-hives in each apiary, composing a lot with a mass of about 500g was sent to the laboratory together with the corresponding data. Macroscopic impurities were removed from each lot. Then, 2-3g subsamples from different parts of lot were

taken for composing the 40g assay sample. These samples, previously cooled at -18ºC for 24 hours, were milled in a refrigerated IKA mill. The resulting product was packaged in glass flasks; 20g were used as a control sample and 20g were left to carry out assays.

Forty-two useful samples were collected; 14 from each of the three already described phytogeographical regions and they were submitted to the following determinations:

a) *Physicochemical characterization*: Our methods are from the recommended IRAM - INTA 15935-1 Scheme 1 (2004), and they are the same techniques used by other authors to characterize similar samples (Woisky y Salatino, 1998; Maidana, 2000; Maldonado, 2000).

Sensory attributes (ISO 6658: 1985), *loss by heating* (in oven at 100ºC $\pm$ 2ºC, until constant weight), *ash* (in a muffle furnace at 550ºC $\pm$ 25ºC, until constant weight), *n-hexane extractable substances* (by means of n-hexane extraction in a reflux Soxhlet extractor), *resins soluble in ethanol* (by means of ethanol extraction in a reflux Soxhlet extractor on the solids residue obtained after determining the n-hexane extractable substances) *insoluble residue* (represented by dry solids residue obtained after determining the ethanol soluble resins), *oxidation value* (discoloration time of 0.05ml potassium permanganate solution 0.1N, by 2ml of hydroalcoholic 0.2% propolis solution), *UV absorption spectrogram* (by means of scanning spectrophotometric reading between 240nm and 420nm of the absorbance of an alcoholic dilution 1:1000 of ethanolic extract obtained from the soluble resins determination; Spectrophotometer Varian Cary 50), *total phenolic compounds* (by spectrophotometry; measuring at 765nm, the absorbance of

a dilution 1:200 of ethanolic extract obtained from the determination of soluble resins, to which a Folin-Ciocalteu reagent is added and left to react 5 minutes at 50ºC). The method used for determining total phenolic compounds is limited because of its low specificity towards certain polyphenols that may be present (Mosca, 2000) and the total phenolic compounds are only estimated.

Two-way ANOVA were applied to determine the effects of the phytogeographical areas on the physico-chemical characteristics. Post-hoc comparisons were made by least significant difference (LSD) test.

b) *Complementary characterization studies*: as colorimetric methods low-specificity for phenolic compounds, some of them were identified and quantified by using HPLC. This compounds share a skeleton of dyphenil pyrans (C6-C3-C6), formed by two phenyl rings bounded by a C pyran ring, consider profit by their antioxidant capacity (Martínez Flores et al., 2002). A high performance liquid chromatographic (HPLC) procedure has been used to identify and determine some flavonoids in propolis (Bankova et al., 1982). A Shimadzu LC 10-AS chromatograph, equipped with a UV-visible SPD-10A detector and a C 18 reversed-phase column (Supelco), particle size 5µm, 25x0.46cm ID and pre-column (4mm) of the same material. The eluent was water-methanol-acetic acid (60:75:5, HPLC quality), at a flow rate of 0.7ml/min. Standard solutions were prepared by dissolving hydroxycinamic acids (cafeic acid, o-cumaric acid, p-cumaric acid), benzoic (syringic acid) and cynamic (cumaric); flavons (apigenine, acacetine, crisine), flavonols (quercetine, kaempferol, galangine); provided by SIGMA Chemical Company. Sample for the HPLC analysis involved dilutions of balsams

obtained in the determination of ethanol-soluble resins, applying the methods proposed by Markaham et al. (1996).

RESULTS AND DISCUSSION

Most propolis (64%) was dark brown or greenish irregular pieces, with intense but very aromatic resin odour. Less than 15% of the samples were a bright uniform mass; 59% of the samples presented a hot spicy or hot sweet taste.

The results obtained are given in Table I.

Table I – Physico-chemical characteristics of propolis produced in Santa Fe

Area		loss by heating	ash	n-hexane extractable substances	insoluble residue	ethanol soluble resins	total phenolic comp.	oxidation value
	Units	g/100g	g/100g	g/100g	g/100g	g/100g	g/100g	s
Pampeana	Minimum	1.4	1.8	14.3	18.0	26.6	11.3	14
	Maximum	1.6	2.2	46.2	22.4	75.8	22.8	19.5
	Median	*1.5*	*2.0*	*33.2*	*20.5*	*61.2*	*21.3*	*16*
Espinal	Minimum	1.5	1.9	22.1	18.5	29.0	11.4	12
	Maximum	1.7	2.3	28.4	21.4	55.3	21.3	24
	Median	*1.6*	*2.0*	*27.1*	*20.0*	*40.1*	*17.2*	*18*
Chaqueña	Minimum	1.7	1.8	17.6	18.5	38.8	11.3	2
	Maximum	6.2	2.4	30.6	29.2	55.0	38.0	48
	Median	*4.5*	*2.2*	*28.2*	*21.8*	*41.9*	*16.6*	*21*

Samples from *chaqueña province* have the highest values of loss by heating and differ from those of the other areas (p=0.05, LSD test).

Resins content soluble in ethanol are significantly highest (p=0.05, LSD test) in *pampeana province* samples; in coincidence with higher total phenolic compounds content.

Total phenolic compounds content in pampena province propolis agree with those reported by Woisky and Salatino (1998). Concentrations corresponding to phenolic and flavonoid compounds obtained by HPLC (Table II) show the predominance of pinocembrin, crisine, galangine and quercetine in these samples. Both antibacterial flavonoid pinocembrin and antiviral crisina are present in all the samples. Bedascarrabure et al. (2003) reported that total flavonoids content in pampeana province propolis is 7.87% $\pm$ 0.39 and in chaqueña province samples is 3.61% $\pm$ 0.40.

UV spectrograms show absorbance peaks at 240nm, 270nm and 340nm.

Table II – Concentration (g/100g) of some active Phenolic and Flavonoid compounds in Propolis collected in Santa Fe (Argentine)

		Concentration Ranges
Phenolic Compounds	Cafeic acid	Not detectable - 0.18
	Cumaric acid	0.13 – 0.56
	Syringic acid	0.06 - 2.23
Flavonoids	Quercetine	1.83 - 5.13
	Apigenine	Not detectable - 2.30
	Kaempferol	Not detectable
	Galangine	Not detectable - 8.85
	Acacetine	Not detectable
	Crisine	2.33 - 7.46
	Pinocembrine	2.10 - 10.00

CONCLUSIONS

This analysis suggests that propolis in the Province of Santa Fe differ in their phenolic compounds. The highest phenolic compound content was found in

propolis from the *pampeana province* where the mirthaceas and salicaceas species grow.

If physico-chemical specification for raw propolis are performed as stated by the Argentine Standardization Institute (Instituto Argentino de Normalización - IRAM) to be applied within this country, 20% samples exceed the highest level established for n-hexane substances extractable (≤35%) and 5% samples exceed the maximum oxidation value (≤22s).

However, further sample analyse should be carried out to confirm these features.

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