PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE
FACULTAD DE AGRONOMÍA E INGENIERÍA FORESTAL

CERTIFICATION OF HONEY ANALYSIS: BOTANICAL ORIGIN, PESTICIDES, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY

Laboratorio de Botánica y de Productos Naturales
Facultad de Agronomía e Ingeniería Forestal

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Introduction

The honey bee (*Apis mellifera*) use the nectar produced by plants to make honey and maintain in a sustainable way the hive. Honeybees are selective when using the plant species that are available in the surrounding of the beehives, selecting those that produced a nectar of better quality (greater volume and an adequate concentration of sugars, and free of alkaloids). When honeybee collects the nectar from the flower, get contaminated with their pollen grains, transporting them from one flower to another and producing the pollination. Part of that pollen falls on the nectar that the bee collects, so it will appear in the honey that is made off. Pollen grain are specific, because shape and fine structure are genetically codified, so the identification and analysis of pollen frequencies in honey, allows to determine its botanical and geographical origin, then called honey according to that origin.
Due to honey selectivity is that not all honeys are equal, inheriting different secondary compounds from the plant species that comprise it, acquiring antimicrobial and antioxidant properties, among others.

Materiales

The honeys analyzed corresponded to 1 sample of honey, harvested during the March 2018 season. The samples analyzed are shown in Table 1. Samples were sent to the Laboratorio de Botánica of Facultad de Agronomía e Ingeniería Forestal of the Pontificia Universidad Católica de Chile. The analysis was requested by Honey Group Chile.

Table 1.- List of analyzed samples, with the information of the beekeeper that obtained them and their localities of origin.

	Sample	Weight (Kg)	Producer	Region	Location	Year production
1	M1880	1	Honey Group Chile	X	Chiloé	March 2018

Analysis

I. Botanical Origin

Method:

The analysis was carried out following the protocol indicated in Chilean Standard NCh 2981-2005 " Denominación de Origen Botánico mediante ensayo Melisopalinológico", which is the official standard norm for the determination of Botanical Origin of honey in Chile.

Table 2.- List of samples analyzed, with the number of pollen morphs found for each, the main species with its percentage of participation and the secondary species with its percentage of participation.

Sample	N° species found	Main specie	Percentage (%)	Secondary specie	Percentage (%)
M1880	7	*Eucryphia cordifolia*	66,61	*Lotus pedunculatus*	19,72

Results

The honey analyzed correspond to the following categories, according to the classification of Chilean Standard NCh Nº 2981-Of.2005:

Table 3. - Classification of honeys analyzed according to the Botanical origin.

	Sample	Producer	Clasification
1	M1880	Honey Group Chile	Monofloral Nativa of ulmo (*Eucryphia cordifolia*)

Honey M1880 corresponds to a sample of honey **Monofloral Nativa of ulmo (*Eucryphia cordifolia*)**, i.e. unifloral hney of ulmo, because this species is predominant in the honey with respect to other plant species that are present, reaching percentages of participation of 66,61% in the sample.

The ulmo is an evergreen tree with simple leaves, of solitary flowers located in the upper part of the branches. It is characterized by being a white flower with 4 free petals and many stamens. It produces a large amount of nectar, appearing frequently in honeys produced from the Araucanía to the Los Lagos Region.

II. Determination of antimicrobial activity

Pathogenic bacteria used to control biocide of honey.
The pathogenic bacteria against which the effect of the extracts was tested corresponded to *Escherichia coli*, *Pseudomona aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes*, from the stock of the Natural Products Laboratory at the University. The bacteria were activated one day prior to their use in a soybean agar culture medium. The next day a bacterial inoculum was prepared in physiological saline at a concentration of 106 CFU, according to the McFarland scale.

Method: Well Diffusion Agar (WDA)
To carry out the WDA test, we proceeded as follows:
Petri dishes were sown in the form of grass. Once planted, 3 pockets were made in the culture medium with a punch. In one of them, a drop of 4% phenol was placed, and in the other two a drop of honey: water was placed 1: 1, and they were left in the growth chamber for a time of 48 hrs. After the time the plates were checked and it was verified in which there was inhibition of the growth of the bacteria, measuring the formation of a halo around the place where the drop was placed, showing the scope that had the inhibition of the bacterial development (halo of inhibition of growth).

Table 4. - Average measurement in mm of the inhibition halo for each bacterium used.

	Inhibition halo (mm) for each bacterium			
Sample	*Streptococcus pyogenes*	*Pseudomona aeruginosa*	*Staphylococcus aureus*	*Escherichia coli*
M1880	8,00	19,00	11,33	14,67

III. Determination of Antioxidant Activity

Method: FRAP
The Antioxidant Activity was determined by the FRAP method, which consists in analyzing the ability of plasma to reduce ferric iron. Absorbance was measured using a spectrophotometer (Shimadzu UV-160, Kyoto, Japan) at 593 nm. The results were expressed as millimoles of Trolox per kg of honey (Trolox mmoles / kg of honey).

Sample	**Result**	**Unity**
M1880	1,1	mmol Trolox/kg miel

Tabla 5. Values obtained through the evaluation in a spectrophotometer.

The Reference Values were used in arámdano whose antioxidant activity is the highest obtained in nature.

- FRAP: 14,2 mmol Trolox/kg arándano

These reference values were obtained from an average variety of cranberry fruits grown in Chile.

IV. Residues of Pesticides Neonicotinoides

Method

For the determination of Neonicotinoid residues in honey, solid phase extraction (SPE) and quantification by high efficiency liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS / MS) was implemented.

	Pesticide Concentration (µg / Kg miel)			
Sample	**Imidacloprid**	**Thiamethoxan**	**Acetamiprid**	**Thiacloprid**
M1880	<LOD	<LOD	<LOD	<LOD

Table 7.- Concentration of pesticides found in honey, measured in µg / Kg honey. (LOD= Limit of detection)

This certificate is issued in the name of **Honey Group Chile.**

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Anexes

Table 5.- Total pollen grains and corresponding percentage of species found in the pollen fraction of honey M1880.

M1880					Rango 95% confianza	
Especie	**NC**	**n° granos**	**Porcentaje**	**+/-**	**Min**	**Max**
Trifolium repens	trébol blanco	3	0,533	0,601	-0,069	1,134
Hypochaeris radicata	hierba del chacho	8	1,421	0,978	0,443	2,399
Eucaliptus sp.	euclaipto	11	1,954	1,143	0,811	3,097
Luma apiculata	arrayán	20	3,552	1,529	2,023	5,081
Myrceugenia planipes	pitra	35	6,217	1,995	4,222	8,211
Lotus pedunculatus	alfalfa chilota	111	19,716	3,286	16,429	23,002
Eucryphia cordifolia	ulmo	375	66,607	3,896	62,712	70,503
Total		563	100,000			

Figure 1.- Participation percentage chart, showing the species whose pollen was detected in the honey sample M1880.