BEHIND OF

“ACTIVE PATAGONIA FACTOR”

Certified antibacterial honey from Chilean Patagonia

This book shows more than 10 years of scientific research on Chilean native honeys carried out by the prestigious Catholic University of Chile. The result is the creation of the APF factor, which certifies antibacterial properties in honey with unique medicinal characteristics.

*Developed by JPM Exportaciones Ltda

INDEX

"BEHIND OF APF FACTOR"

INTRODUCTION

BEHIND OF APF FACTOR RESEARCHES:

1) 2008 - Application of the Chilean Official Standard to designate the botanical origins of honey for the characterization of the apicultural production.
2) 2009 - Antibacterial and antifungic activity of the unifloral honeys of Quillaja saponaria, an endemic Chilean species
3) 2009 - Nectar volume and floral entomofauna as a tool for the implementation of sustainable apicultural management plans in Quillaja saponaria
4) 2009 - Analysis of volatile compounds in three unifloral native Chilean honeys
5) 2010 - Multivariate analysis of pollen frequency of the native species Escallonia pulverulenta (Saxifragaceae) in Chilean honeys
6) 2011 - The medicinal value of honey: a review on its benefits to human health, with a special focus on its effects on glycemic regulation
7) 2012 - The antioxidant activity of Chilean honey and bee pollen produced in the Llaima volcano's zones
8) 2013 - Antioxidant and antimicrobial activity of unifloral honeys of plants native to Chile.
9) 2013 - Biological applications of Chilean honeys produced by Apis mellifera
10) 2013 - Phenolic profiles of nectar and honey of Quillaja saponaria as potential chemical markers
11) 2013 - Volatile compounds of unifloral honey and floral nectar from Quillaja Saponaria
12) 2014 - Comparison of methods for determining the color of Chilean honeys and the relationship of color with botanical origin in central Chile
13) 2014 - The importance of weeds as melliferous flora in central Chile
14) 2016 - Nectary structure in four melliferous plant species native to Chile
15) 2017 - Differences between phenolic content and antioxidant capacity of quillay Chilean honeys and their separated phenolic extracts
16) 2017 - Relation between composition, antioxidant and antibacterial activities and botanical origin of multi-floral bee pollen
17) 2018 - Bioactive polyphenols and antioxidant capacity of Azara petiolaris and Azara integrifolia Honeys
18) 2019 - Rapid Scanning of the Origin and Antioxidant Potential of Chilean Native Honey Through Infrared Spectroscopy and Chemometrics
19) 2019 - The Value of Chilean Honey: Floral Origin Related to their Antioxidant and Antibacterial activities.
20) 2010 - Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant Staphylococcus aureus and Escherichia coli. Made by BMC Complementary and Alternative medicine

APPENDIX 1.0: Analysis examples of Chilean honey against 3 bacteria Escherichia coli, Staphylococcus aureus, Salmonella enterica.

APPENDIX 2.0: Chilean beekeeping photos

INTRODUCTION

Gloria Montenegro Rizzardini is a botanist, biologist, academic and scientist. She is Professor of Botany at the Pontificia Universidad Católica de Chile. She won the L'Oréal-UNESCO Awards for Women in Science in 1998. She has undertaken pioneering work in botany and conservation of native flora, using scientific approaches to protect plant ecosystems.

The research team, led by Gloria Montenegro, carried out different science, research, and technology and development projects over more than three decades.

These generated results provisionally called "analysis of the biological activity of various native/endemic Chilean honeys concerning its biocidal potential". (Lusby et al. 2005;Muñoz et al 2007; Lee et al. 2008; Montenegro & Ortega 2011; Schencke et al. 2011;Montenegro et al. 2013a; Montenegro et al. 2013b; Calderon et al. 2015; Schencke et al.2015; Bridi et al. 2016; Montenegro & Velasquez 2017, Bridi & Montenegro 2017;Giordano et al. 2018)

According to the Codex Alimentarius, honey is a natural sweet substance produced by bees (Apis mellifera) from flower nectar or secretions of living parts of the flowers. The nectar is collected and transformed into honey by enzymatic actions and dehydration, reaching water content of approximately 18% (Montenegro and Mejias, 2008). Honey contains proteins, organic acids, vitamins (especially vitamin B6, thiamine, niacin, riboflavin and pantothenic acid), minerals (including calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc), pigments solid particles derived from bee pecoreo. Besides a great variety of volatile compounds and secondary compounds derived from the plant species from which it originates, such as phenolic compounds and terpenes. Chemical, sensory, physical and microbiological characteristics are the main determinants of honey quality (Alvarez-Suarez et al., 2010, Montenegro et al. 2013).

One of the main reasons for the antibacterial activity of honey is its high sugar content (close to 80%) and acid pH (between 3 and 4.5) due to gluconic acid, derived from glucose catalysis (Araya, 2004; Ordoñez, 2015, Ramírez, 2013; Cortez et al. 2011, Ulloa, 2010). Also, when bees visit the flowers, they introduce an enzyme to convert the nectar into honey. This enzyme, called hydrogen peroxide activity (HPA), gives it antibacterial properties (Garcia, 2018; Ramirez, 2013; Ordonez, 2015). A study published in 1937, was the first to examine in detail the honey's antimicrobial effect and its authors called "inhibin", which was initially responsible for this effect, before identifying it as hydrogen peroxide. It is important to note that most varieties of honey contain hydrogen peroxide when freshly harvested from the hive. However, this enzyme is thermolabile and photosensitive, so if it is excessively heated or exposed to direct light for a long time in its packaging or storage, it loses its antiseptic properties (Moore, 2011).

In bee honey, there are also non-peroxide type compounds that generate antibacterial activity; these vary according to the plant species from which the bees take the nectar (García, 2018). We identified the defensin-1 peptide, attributing it antimicrobial properties after studies carried out with several microorganisms (Fiorilli, 2015).

As the honey inherits properties, according to its botanical origin, one would expect to find significant differences in its biological activity. Worldwide, the honey of Eucalyptus marginata, Kunzea ericoides,

Leptospermum scoparium and Knightea excelsa have high HPA levels and therefore used in many cures (Alqarni, 2012, Stephens et al., 2010).

In Chile, the botanical origin of honey has been studied, allowing its export with a certificate of botanical and geographical origin, while demonstrating, at the same time, the variety of native flora used by bees (Ramirez and Montenegro, 2004, 2000; Montenegro et al., 2003, 1992; Avila et al., 1993; Montenegro and Avila, 1995). Thus, among Chilean varieties of honey, the botanical origin of endemic species such as "Corontillo" Escallonia pulverulenta and "Quillay" Quillaja saponaria in the central and north-central zone of the country and native species such as "Ulmo" Eucryphya cordifolia, in the southern region, stands out (Montenegro, 2002; Montenegro et al., 2003; Montenegro et al., 2008; Montenegro et al., 2010a; Montenegro et al., 2010b). Studies conducted by the Pontificia Universidad Católica de Chile indicate that the different native honey of Chile show significant differences in both antioxidant activity and activity against pathogens, which depends on the botanical and geographical origin and may be associated with the content of polyphenols (Montenegro et al. 2012).

Different studies state that there is a close relationship between the floral origin vs. color vs. composition vs. antioxidant capacity vs. antimicrobial capacity of the bee honey, where the different darkest honey are the ones that reflect a higher content of bioactive compounds and therefore a higher biological activity. In a 2010 study, I observed significant antimicrobial activity for ulmo and Manuka UMF 25+. The scientists were amazed by the ulmo's activity and suggested to study it in depth. That is why ulmo single-flower honeys have been used for research in wound healing based on their proven antibacterial properties. Sherlock et al., (2010) tested the antimicrobial activity of Chilean honey produced from ulmo against a group of bacteria, showing this honey has a higher antibacterial effect for Staphylococcus aureus, Escherichia coli and Pseudomonas aeroginosa compared to Manuka honey (Schencke, 2013).

It is worth mentioning that recent studies indicated that dilutions of native Chilean ulmo honeys had an inhibitory action at different concentrations against bacteria such as Pseudomona aeruginosa and Escherichia coli. However, this did not occur in all ulmo honey, which could be because the antimicrobial characteristics of diverse kind of honey are affected, among other things, by the accompanying species, which vary according to the year and area, which would explain the differences between samples of the same botanical origin (Kucuk et al., 2007; Molan, 1992).

Several works by Montenegro and collaborators have proven the bactericidal properties of Chilean honeys that originates from native or endemic species of the temperate forest of southern Chile, the scrublands of central Chile and the shrubby steppes of the northern zone (Muñoz et al. 2007; Montenegro & Ortega 2011; Montenegro et al. 2013a; Montenegro et al. 2013b; Bridi et al. 2016; Montenegro & Velasquez 2017, Bridi & Montenegro 2017; Giordano et al. 2018;).

Chile has a great diversity of endemic and native plant species that can give rise to a range of honey produced by Apis mellifera. Continental Chile has an area of nearly 75 million hectares located in the southwest of South America. It has a length of approximately 4,300 kilometers from north to south and an average width of 180 kilometers. The presence of two mountain ranges, such as the Andes and the Coastal Range, generate very diverse geomorphology with very different valleys and microclimates, besides a diverse community of plants capable of producing nectar for Apis mellifera. Also, the presence of the Atacama Desert to the north and the Pacific Ocean to the west determines geographical isolation evolving towards biodiversity characterized by high endemism of species and ecosystems. Chile's vascular flora has just over 5,500 species, not including subspecies and varieties (Marticorena, 1990, Montenegro 2000; Montenegro et al 2002). Although the number of species compared to other Latin American

countries is low, the most prominent feature is that almost half of the flora is endemic, giving a unique character to the products that arise from these species, such as "bee honey" which also result in unique biological properties. (Montenegro y Mejias 2013; Montenegro & Ortega 2011 WO/2011/057421; Montenegro y Mejias 2013; Silva et al 2016; Bridi et al. 2016; Montenegro & Velázquez 2017; Bridi & Montenegro 2017; Giordano et al. 2018). Central Chile is a biodiversity hotspot where endemic plants account for 0.5% of the 300,000 plant species described worldwide. This means that 46.8% of the plants described in Chile are endemic (Myers et al., 2000).

The production of single-flower honeys of endemic or native floral origin arises in two large geographical regions of the country. The first corresponds to the Central Zone of Chile, which has a semi-arid Mediterranean climate, where the Evergreen Sclerophyll Scrub is the dominant vegetation. The second, towards the south, corresponds to the humid Mediterranean region where the Temperate Broadleaf Forest, also locally called Bosque Valdiviano, predominates. The characteristic of the central zone is the production of endemic single-flower honeys (Montenegro et al. 2008, Muñoz et al. 2007; Montenegro & Ortega 2011; Montenegro et al. 2013a; Montenegro et al. 2013b; Bridi et al. 2016; Montenegro & Velasquez 2017, Bridi & Montenegro 2017; Giordano et al. 2018) such as quillay (Quillaja saponaria), corontillo (Escallonia pulverulenta) corcolén (Azara petiolaris) and Tevo (Retanilla trinervia). While towards the south we find production of native single-flower honey of hazelnut (Genuina avellana), ulmo (Eucryphia cordifolia), and tineo (Weinmannia trichosperma) and tiaca (Caldcluvia paniculata). Besides, there is a production of honeys from the nectar of introduced species (Muñoz et al. 2007; Montenegro et al. 2009a; Montenegro et al. 2009b; Montenegro et al. 2013). The pollen profile in the honey reflects the vegetation surrounding the apiary, the seasonal floral diversity, and the species composition of the plants the bees have foraged. The relative frequency of pollen grains present in honey is determined through the melissopalynological method internationally accepted as an indicator of botanical/geographical origin, a tool of great importance for marketing honey with higher added value (Montenegro et al. 2010; Corvucci et al. 2015). In Chile, the official standard (NCh2981.Of2005, Montenegro, et al. 2008) establishes that the melissopalynological analysis must be used to differentiate the botanical origin of the diverse kind of honey produced in this country, which can classify according to three different types of floral or botanical sources: mono-flower, bi-flower, or poly-flower. Single-flower or uni-flower kinds of honey are those where at least 45% of pollen or more belongs to a particular species. Bi-flower honey is that where the pollen of two species is dominant, covering a total between both of more than 50%. There is not a difference higher than 5% between both. Finally, poly-flower honey is the one where no species covers the previous requirements (Montenegro et al. 2008).

Antimicrobial Analysis

Several studies have proven the antibacterial activity of honeys (Lusby et al. .2005Muñoz et al. 2007; Lee et al. 2008; Montenegro & Ortega 2011; Schencke et al. 2011; Montenegro et al. 2013a; Montenegro et al. 2013b; Calderon et al. 2015; Schencke et al. 2015; Bridi et al. 2016; Montenegro & Velasquez 2017, Bridi & Montenegro 2017; Giordano et al. 2018) describing their bacteriostatic or bactericidal activity. The antimicrobial nature of honey depends on different factors that act individually or synergistically, being one of the most significant, the presence of phenolic compounds of hydrogen peroxide, of the pH of honey among the most important. The antibacterial capacity of ulmo honey (Eucryphya cordifolia) has been previously described and patented (Montenegro and Ortega 2011, Montenegro et al. 2013, Sherlock et al. 2010). Moreover, the methanolic extract obtained using Amberlite XAD-2 columns indicates that phenolic compounds play an essential role in this capacity. In vitro trials showed that these extracts were able to inhibit the growth of Escherichia coli, Pseudomona aeruginosa,

Staphylococcus aureus, and Streptococcus pyogenes for which we also determined the minimum inhibitory concentration (Montenegro and Mejias 2013, Montenegro et al. 2013).

Manuka honey derived from the tree or shrub species Leptospermum that grows in New Zealand and eastern Australia has been described as capable of inhibiting about 60 species of bacteria including aerobic and anaerobic, both highly negative and positive. The antimicrobial activity of this honey against Staphylococcus aureus bacteria makes it an essential functional ingredient for wound healing. The potential of this honey to heal wounds has been repeatedly demonstrated not only by its growth-controlling properties of this bacteria but also by maintaining moisture in the wound area and producing a viscosity that acts as a barrier and helps to protect and prevent infection (Lusby et al. 2005). Concerning quillay honeys (Quillaja saponaria), we analyzed their antibacterial and antifungal activity and showed a significant bacyeriotatic activity against the four bacterias: Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, and Pseudomona aeruginosa. Concerning quillay honeys (Quillaja saponaria), we analyzed its antibacterial and antifungal activity and proved a significant bacteriostatic action against four bacteria: Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, and Pseudomona aeruginosa. The antibacterial effect could be due to the phenolic compounds present in the phenolic extract, such as caffeic, coumaric, and salicylic acids, and to the flavonone naringenine and flavonol kaempferol detected by the High-Performance Liquid Chromatography technique.

APF FACTOR DEVELOPMENT

This seal called: "Active Patagonia Factor," whose abbreviation will be APF indicates different levels of biological activity — controlling the growth of 3 pathogenic bacteria (Escherichia coli, Staphylococcus aureus, Salmonella enterica) of Chilean Honeys from the Native Forest and the extreme south of the country.

The seal was created based on the results of scientific experimentation with more than 500 Chilean honeys of single-flower botanical and known origin coming from species of natural plant communities of Central Chile (evergreen sclerophyllous scrub) and plant communities of southern Chile (Rainforest)

In each of the honeys collected from apiaries located in these wild areas, we determined the total growth controlling activity of 3 pathogenic bacteria: Escherichia coli, Staphylococcus aureus, Salmonella enterica following the methodology described below.

Determination of the honey's antimicrobial activity

The antibiotic activity of honey means the ability to eliminate or inhibit the development and growth of some microorganisms, commonly bacteria or fungi.

Evaluating the potential effectiveness of honey requires having a culture isolated from the pathogens, pathogenicity testing, and testing the efficacy of honey control on pathogen development.

To determine the honeys antibiotic capacity (which allowed to design the graduation and to express it in a seal), we used the Agar Diffusion method (Well Diffusion Agar, WDA)

We performed the WDA test as follows: We used the following bacteria: Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC25923), and enteric Salmonella from the Institute of Public Health of Chile.

Initially, we performed an antimicrobial capacity analysis with DM, for which we used Petri dishes with 25 mL of soy agar (BBL TM Trypticase TM soy agar BD [Sparks, MD USA]), as culture medium, which we seeded with sterile swabs immersed in a bacterial solution. For the bacterial solution, we diluted each bacterial species in saline to a concentration of 106 cfu/mL. We made three holes of 6 mm diameter in each plate, where we placed 100 µL of the extract. Each analysis performed in triplicate. We incubated the plates at 37 °C for 24 hours, and we observed if there was inhibition in the growth of the bacteria, which we measured as millimeters of diameter of bacterial growth inhibition.

Besides, we performed the double micro-dilution test in series on ELISA plates (Pontino et al., 2006) to estimate the minimum bactericide concentration. For this purpose, we used 150 µL of soybean broth, 50 µL of bacterial solution (5 x 103 cfu/mL), and 150 µL of honey extract per hole. We incubated the plates at 37°C for 24 hours. Finally, we performed a subculture of the ELISA plate solutions in Petri plates with 25 mL of soya agar.

We expressed the minimum bactericide concentration (MBC) in g of honey/mL of distilled water, which is necessary to cause bacterial death.

Statistical analysis

For the analysis, we used the statistical software Infostat (Di Rienzo et al., 2012), with which we performed variance analysis to determine the existence of significant differences between honeys analyzed for each parameter evaluated. We determined the differences using Tukey's test (p < 0.05), and we calculated correlations between data using Pearson's correlation coefficient.

After these steps, we will select the honeys that show some degree of activity against the bacteria in question.

Factor Determination

The average level of antibacterial activity against these bacteria results in a number added to the factor, thus determining the levels elaborated according to the average diameter of the three bacteria Escherichia coli, Staphylococcus aureus, Salmonella enterica. To determine the factor, we considered working based on all the honeys that have shown some degree of activity against all the bacteria analyzed, specifying a minimum average diameter of 3 bacteria of 13 mm to consider it as honey with honey with anti-bacterial activity. Based on the results and the antibacterial capacities of the honeys with these characteristics, these honeys were divided into 4 groups:

HONEY WITH LOW ANTI-BACTERIAL ACTIVITY
HONEY WITH MEDIUM ANTI-BACTERIAL ACTIVITY
HONEY WITH HIGH ANTI-BACTERIAL ACTIVITY
HONEY WITH VERY HIGH ANTI-BACTERIAL ACTIVITY

The distribution of % of these honeys under the classification made was:

HONEY WITH LOW ANTI-BACTERIAL ACTIVITY	44%
HONEY WITH MEDIUM ANTI-BACTERIAL ACTIVITY	31%
HONEY WITH HIGH ANTI-BACTERIAL ACTIVITY	18%
HONEY WITH VERY HIGH ANTI-BACTERIAL ACTIVITY	7%

For the determination and equivalence of these results to a level of the APF factor, we decided to exclude all honeys with low anti-bacterial activity, so the factor will be determined with three levels, which we named as follows: APF 100+, APF 150+ and APF 200+, directly associated to their anti-bactericidal capacity.

HONEY WITH MEDIUM ANTI-BACTERIAL ACTIVITY	APF 100+
HONEY WITH HIGH ANTI-BACTERIAL ACTIVITY	APF 150+
HONEY WITH VERY HIGH ANTI-BACTERIAL ACTIVITY	APF 200+

PHOTOS OF RESULTS APF ANALYZIS:

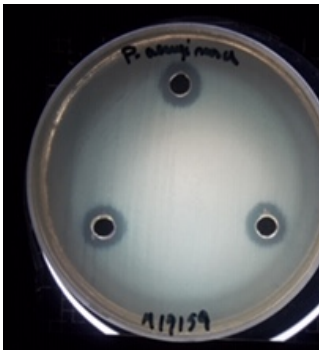
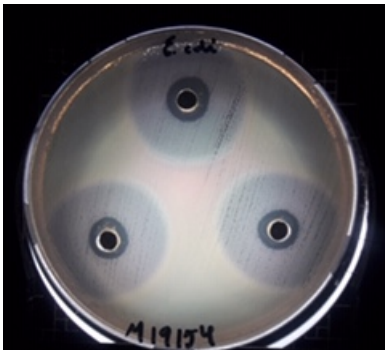
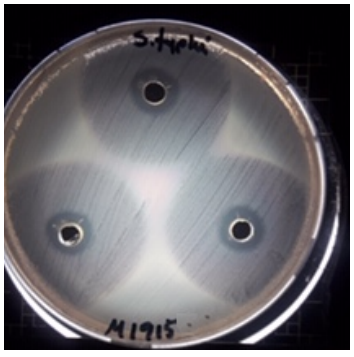
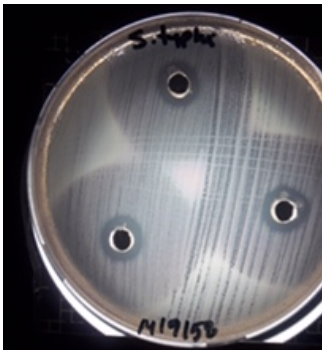

Photo 1: No Anti-bacterial activity level in the honey against bacteria. Result: **NO APF.**
Photo 2: Medium Anti-bacterial activity level in the honey against bacteria. Result: **APF 100+**
Photo 3: High Anti-bacterial activity level in the honey against bacteria. Result: **APF 150+**
Photo 4: Very high Anti-bacterial activity level in the honey against bacteria. Result: **APF 200+**

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ARTICULO DE INVESTIGACION

Aplicación de la Norma Chilena Oficial de denominación de origen botánico de la miel para la caracterización de la producción apícola

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Abstract

G. Montenegro, M. Gómez, J. Díaz-Foriester, and R. Pizarro. 2008. Application of the Chilean Official Standard to designate the botanical origins of honey for the characterization of the apicultural production. Cien. Inv. Agr. 35(2):181-190. Chilean apicultural production is characterized by a great variety of honey types with a high percentage of nectar from native plant species. The proportion of nectar from native plants associated with the high endemism of the Chilean flora results in the production of honeys with special characteristics. Approximately 95% of the honey produced in Chile is exported in bulk without added value and accounts for 1% of the world honey trade. The differentiation of Chilean honeys on the basis of their particular biological origins represents one way to improve their competitive value on the international market. The application of a traceability system and the establishment of the Chilean standard (NCh2881.Of2005), which determines the botanical origin of a given honey by a melissopalynological test, represent two important advances toward differentiation. In order to determine the botanical and geographical characteristics of Chilean honey, 240 honey samples from two consecutive harvesting seasons were studied using the recently approved norm. The results demonstrate that there are two main areas of production. The first area has a Mediterranean climate (Chile's Central zone, 30º to 36º S). The honeys produced there are endemic unifloral and native multifloral, and they represent diverse species. The second area transitions to a humid temperate climate (Central South zone of Chile 36º to 43º S), and the honey produced there includes native and non-native unifloral and native multifloral varieties with restricted botanical diversity.

Key words: *Apis mellifera*, botanical origin, honeybee, Chilean honey, honey characterization.

Introducción

La miel es un producto principalmente demandado por países con un alto nivel adquisitivo. Su comercio mundial alcanzó un valor de 945 millones de dólares y un volumen de 402 mil toneladas en el año 2003. Los principales importadores fueron Alemania y EUA, los cuales adquirieron el 46%. Los principales productores y exportadores mundiales fueron China y Argentina con un 39% de participación en el mercado internacional de este producto (Danty, 2005).

En Chile se producen unas 10.000 t de miel al año (0,8% de la producción mundial), con un consumo interno aproximado de 1.400 t, equivalente a 100 g *per capita* aproximadamente, cantidad inferior al promedio mundial con un consumo *per capita* de alrededor de 220 g al año. Por tanto, la mejor alternativa comercial de la miel chilena es el mercado de exportación (INDAP, 2006).

La Comisión del *Codex Alimentarius* de la FAO/OMS (2001) ha establecido una regulación

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para el comercio internacional de la miel de abejas a través de una norma aplicada en forma íntegra o con algunas modificaciones por los países importadores de este producto. Esta norma establece definiciones y denominaciones y regula los factores esenciales de composición y calidad de este producto.

En Chile, se ha estudiado el origen botánico de la miel, permitiendo su exportación con certificado de origen botánico y geográfico, evidenciando, al mismo tiempo, la variedad de la flora nativa utilizada por las abejas (Ramírez y Montenegro, 2004, 2000; Montenegro *et al*., 2003, 1992; Avila *et al*., 1993; Montenegro y Avila, 1995). Por otro lado, con el fin de determinar el cumplimiento de los requisitos para la exportación de miel, el Servicio Agrícola y Ganadero (SAG, Ministerio de Agricultura, Gobierno de Chile) ha establecido un sistema de trazabilidad de mieles creando el Registro de Apicultores de Miel de Exportación (RAMEX) y el Registro para los Exportadores de Miel (REEM). Además, la División de Normas del Instituto Nacional de Normalización (INN, Chile) estableció en 2005, la Norma Chilena Oficial NCh2981.Of2005 Miel de abejas, Denominación de Origen Botánico mediante ensayo melisopalinológico, con el fin de establecer un método para diferenciar el origen botánico de la miel producida en Chile.

La producción apícola chilena se caracteriza por una gran variedad de tipos de mieles obtenidas de una zona con aptitud melífera de vasta amplitud latitudinal (aproximadamente 16º de latitud sur). Esto se traduce en una amplia variedad climática, vegetacional y en un uso intensivo de la flora nativa como fuente de néctar por *Apis mellifera* otorgándole a las mieles cualidades particulares, debido al alto endemismo de la flora chilena (Montenegro, 2000). Sin embargo, este producto, se exporta principalmente a granel, como miel polifloral, a bajos precios y sólo en algunos casos como miel diferenciada de ulmo (*Eucryphia cordifolia*) o quillay (*Quillaja saponaria*) y en menor cantidad como proveniente de bosque nativo.

Los trabajos realizados sobre denominación de origen y tipificación de las mieles chilenas, se efectuaron antes de la oficialización de la norma chilena respectiva (enero de 2006) y hasta el momento, no existe una tipificación que utilice dicha normativa. Con el fin de caracterizar la producción melífera chilena, en cuanto a su origen botánico y geográfico, se aplicó el método establecido por la norma NCh2981. Of2005 a 240 muestras de miel. Estas muestras se obtuvieron de colmenares ubicados en la zona centro norte, centro y sur de Chile, abarcando siete regiones administrativas (30º a 43º S). En esta vasta zona existen variadas formaciones vegetales nativas, desde el matorral hasta los bosques templados lluviosos del sur del país.

Materiales y métodos

Muestras

Se examinaron los resultados de análisis melisopalinológicos realizados a 240 muestras de miel, provenientes de apiarios ubicados desde la IV a la X Región de Chile y de cosechas realizadas durante las temporadas 2001-2002 y 2002-2003 (Cuadro 1) (Ramírez y Montenegro, 2004; Montenegro *et al*., 2003). Cada muestra se tipificó de acuerdo con su origen botánico y al origen geográfico de las especies que la compusieron, según el procedimiento establecido en la Norma Chilena Oficial NCh2981.Of2005 (Cuadro 2). El análisis se realizó teniendo en consideración el total de las muestras de las dos temporadas.

Análisis melisopalinológicos

En los análisis melisopalinológicos, se consideraron como principales fuentes de néctar sólo aquellas especies cuyo polen reveló una frecuencia estadísticamente superior al resto de las especies que constituyeron la fracción polínica correspondiente.

Tipificación

De acuerdo con la tipificación de las mieles chilenas (norma NCh2981.Of2005), las mieles monoflorales fueron aquellas que procedieron principalmente de una sola especie y en cuya composición polínica se encontró, como mínimo, un 45% de polen de esa especie vegetal. Las mieles poliflorales fueron aquellas elaboradas a partir del néctar de varias especies

Cuadro 1. Número de muestras de miel analizadas, desglosadas por regiones y temporadas de cosecha de la miel en Chile.
***Table 1**. Number of honey samples analyzed, by regions and seasons of harvest in Chile.*

Región administrativa de Chile	Número (no.) de muestras en las temporadas de cosecha de miel		Total no.
	2001-2002	2002-2003	
IV	15	0	15
V	12	6	18
RM	6	27	33
VI	11	20	31
VII	18	1	19
VIII	8	25	33
IX	19	53	72
X	10	9	19
Total	99	141	240

y en cuya composición polínica el polen de ninguna de ellas alcanzó un porcentaje igual o superior al 45%. Se consideraron mieles biflorales, aquellas en cuya composición polínica se encontró significativamente polen de dos especies (ambas ≥ 45%) y en proporción similar (con una diferencia < 5%).

Las mieles mono, bi y poliflorales se clasificaron también de acuerdo con la norma, según el origen geográfico de las especies de las cuales provinieron, siendo endémicas (especies vegetales con una distribución restringida sólo a Chile), nativas (especies vegetales nativas de Chile, pero también presentes en forma natural en otros países), no nativas (especies vegetales que han sido traídas a nuestro país de forma accidental o deliberada) o mixtas (especies vegetales nativas e introducidas). Por lo tanto, las mieles monoflorales fueron endémicas, nativas o no nativas si la especie que aportó con el 45% o más de la fracción polínica de la miel fue endémica, nativa o introducida, respectivamente. Las mieles biflorales se consideraron endémicas o nativas, si las dos especies que le otorgaron ese carácter pertenecieron a alguna de estas categorías y se consideraron mieles mixtas si una especie fue nativa y la otra introducida. Las mieles poliflorales se consideraron nativas o no nativas si las especies correspondientes lograron un porcentaje de participación igual o superior al 45% de la fracción polínica y se consideraron mieles mixtas cuando ninguna especie tuvo una participación igual o superior al 45% de la fracción polínica.

Diseño y análisis estadísticos

El análisis estadístico de los resultados melisopalinológicos se realizó mediante análisis de proporciones clásico, calculando el máximo estimador verosímil, con un 95% de confianza (Mead *et al*., 1993).

Cuadro 2. Tipificación compuesta de mieles chilenas según flora melífera y clasificación según especie(s), de acuerdo con la Norma Chilena Oficial NCh2981.Of2005.
***Table 2.** Classification of Chilean honeys according to their botanical origin (Chilean Official Standard NCh2981. Of2005).*

Clase de miel	Tipo monofloral	Tipo bifloral	Tipo polifloral
Endémica	Monofloral endémica	Bifloral endémica	Polifloral endémica
Nativa	Monofloral nativa	Bifloral nativa	Polifloral nativa
No nativa	Monofloral no nativa	Bifloral no nativa	Polifloral no nativa
Mixta		Bifloral mixta	Polifloral mixta

Resultados y discusión

La aplicación de la Norma Chilena Oficial (NCh2981.Of2005) dio como resultado la tipificación de las mieles, de acuerdo con su origen botánico, en monoflorales (71 muestras), biflorales (24 muestras) y poliflorales (145 muestras) (Cuadro 3).

Las mieles monoflorales endémicas fueron originadas por dos especies, las monoflorales nativas por tres especies y las monoflorales no nativas por seis especies (Cuadro 4).

La producción de mieles monoflorales endémicas y nativas se segregaron en dos grandes áreas geográficas: la primera correspondiente a las regiones V y Metropolitana productoras de mieles monoflorales endémicas de quillay (*Q. saponaria*) y corontillo (*Escallonia pulverulenta*:) y la segunda correspondiente a las regiones VIII, IX y X, donde se obtuvieron mieles monoflorales nativas de avellano (*Gevuina avellana*), ulmo (*Eucryphia cordifolia*) y tineo (*Weinmannia trichosperma*) (Cuadro 4).

Las regiones de la primera área geográfica pertenecen a la zona de clima mediterráneo de Chile (di Castri y Hajek, 1981) y las especies utilizadas por *A. mellifera* como la principal fuente de néctar para elaborar este tipo de miel, son un elemento importante de comunidades naturales como el matorral esclerófilo y el bosque esclerófilo, presentes en esta zona. Los rangos de distribución geográfica de las dos especies se extienden desde la IV a la VII Región para el quillay y desde la IV a la IX Región para el corontillo (Rodríguez *et al*., 1983). Hacia el sur del rango de distribución de estas especies se obtuvo sólo una miel monofloral de quillay (VII Región) y su polen apareció principalmente formando parte de la fracción poliníca de mieles poliflorales nativas y mixtas, y originó la única miel bifloral endémica en un apiario ubicado en la VI Región de Chile. Además de la abundancia de las especies vegetales antes mencionadas, estos resultados se podrían relacionar con el

Cuadro 3. Tipificación de las muestras de miel analizadas, según la Norma Chilena Oficial NCh2981.Of2005, separadas por temporada de cosecha y región. En las columnas correspondientes a las regiones de Chile, se indican el número de muestras de cada tipo de miel (para la IV Región sólo se contó con muestras de la temporada 2001-2002).
***Table 3**. Classification of the analyzed honey samples according to the Chilean Official Standard NCh2981.Of2005. They are grouped by harvesting season and by geographical region, and the columns indicate the number of samples of each type of honey (samples at region IV correspond only to season 2001-2002).*

Tipos de miel[1]	Temporada años	Número (no.) de muestras de miel por regiones administrativas de Chile								Total no.
		IV	V	RM	VI	VII	VIII	IX	X	
Mieles monoflorales nativas	2001-2002	0	4	1	0	0	0	1	2	8
	2002-2003		0	6	0	1	3	1	2	13
Mieles monoflorales no nativas	2001-2002	3	0	0	0	1	0	6	0	10
	2002-2003		0	2	5	0	3	27	3	40
Mieles biflorales nativas	2001-2002	0	0	0	3	0	0	0	0	3
	2002-2003		0	0	0	0	0	0	0	0
Mieles biflorales no nativas	2001-2002	0	1	0	0	1	0	2	1	5
	2002-2003		0	1	0	0	4	2	0	7
Mieles biflorales mixtas	2001-2002	1	0	0	0	1	0	0	0	2
	2002-2003		1	2	1	0	0	1	2	7
Mieles poliflorales nativas	2001-2002	5	4	2	1	4	0	2	3	21
	2002-2003		0	5	4	0	0	3	2	14
Mieles poliflorales no nativas	2001-2002	4	1	2	6	8	7	7	4	39
	2002-2003		4	6	9	0	12	15	0	46
Mieles poliflorales mixtas	2001-2002	2	2	1	1	3	1	1	0	11
	2002-2003		1	5	1	0	3	4	0	14
Total		15	18	33	31	19	33	72	19	240

[1] Las mieles endémicas están incluidas en las nativas.
[1] The endemic honeys are including in native honeys.

Cuadro 4. Número de mieles monoflorales producidas durante las temporadas 2001-2002 y 2002-2003 desde la IV a la X Región de Chile.
Table 4. *Number of unifloral honeys produced during seasons 2001-2002 and 2002-2003 from regions IV to X of Chile.*

Mieles monoflorales	Número (no.) de muestras de miel por regiones administrativas de Chile								Total no.
	IV	V	RM	VI	VII	VIII	IX	X	
Endémicas									
Corontillo (*Escallonia pulverulenta*)		2	1						3
Quillay (*Quillaja saponaria*)		2	6		1				9
Nativas									
Avellano (*Gevuina avellana*)						3			3
Ulmo (*Eucryphia cordifolia*)							1	4	5
Tineo (*Weinmannia trichosperma*)							1		1
No nativas									
Alfalfa (*Medicago sativa*)	1			5		3			9
Yuyo (*Brassica rapa*)	2		1						3
Lotera (*Lotus uliginosus*)			1				31	2	34
Zarzamora (*Rubus ulmifolius*)					1				1
Trevul (*Melilotus indicus*)							1		1
Hierba azul (*Echium vulgare*)							1	1	2

manejo y la ubicación de los apiarios y con la época de cosecha de la miel, ya que muchos apicultores privilegian la producción de mieles monoflorales para lograr un mejor precio. De esta manera, la Zona Central de Chile se podría considerar un centro de producción de mieles endémicas con denominación de origen. Así se avanzaría en el proceso de diferenciación de las mieles chilenas para convertirlas en productos de mayor valor y prestigio internacional, como sucede, por ejemplo, con la miel de Manuka obtenida a partir del néctar de *Leptospermum scoparium*, endémica de Nueva Zelanda. Este tipo de miel tiene un valor comercial 7 a 10 veces superior al de una miel normal para consumo alimenticio.

La segunda área corresponde a una región de transición climática que va desde un clima mediterráneo húmedo (VIII Región) a uno templado húmedo (X Región) (di Castri y Hajek, 1981). Las comunidades vegetales nativas están representadas principalmente por bosques caducifolios, esclerófilo y valdiviano. El ulmo, el avellano y el tineo son especies nativas de Chile y Argentina, que forman parte de estas comunidades vegetales con una distribución circunscrito a los bosques subantárticos, los cuales ocupan ambas vertientes de la Cordillera de Los Andes a partir de los 39º S. Por lo tanto, corresponden a especies con un endemismo regional, otorgándole a este tipo de miel el carácter de endémicas de la región. La miel de ulmo es un producto conocido en el mercado chileno que alcanza mejores precios que las mieles poliflorales. Es fácilmente reconocible por su color blanquecino y su aroma floral (jazmín) anisado. Muchos apicultores trasladan sus apiarios a la X Región de Chile, durante los meses de enero y febrero, para producir este tipo de miel monofloral. Por lo tanto, esta región tiene un gran potencial como zona productora de miel diferenciada con denominación de origen.

Las mieles monoflorales no nativas se concentraron en la IX Región (Cuadro 4). Las especies que las originaron fueron todas herbáceas (a excepción de zarzamora *Rubus ulmifolius*) las cuales cubren grandes extensiones de terreno, ya sea como malezas de campos de cultivos o bordes de camino (*R. ulmifolius, Melilotus indicus*, *Echium vulgar*e) o formando parte de grandes extensiones de praderas cultivadas para forraje (*Medicago sativa*) o para pasturas (*Lotus uliginosus*). Esta última originó 31 de las 33 muestras de mieles monoflorales provenientes de la IX Región de Chile.

Cuadro 5. Número de mieles biflorales producidas durante las temporadas 2001-2002 y 2002-2003 desde la IV a la X regiones de Chile.
Table 5. *Number of bifloral honeys produced during seasons 2001-2002 and 2002-2003 from regions IV to X of Chile.*

Mieles biflorales	Número (no.) de muestras de miel por regiones administrativas de Chile								Total no.
	IV	V	RM	VI	VII	VIII	IX	X	
Endémicas									
Quillaja saponaria- Escallonia pulverulenta					3				3
No nativas									
Eucalyptus globulus-Brassica rapa		1							1
Medicago sativa-Prunus sp.			1						1
Eucalyptus globulus-Prunus sp.					1				1
Medicago sativa-Echium vulgare						2			2
Medicago sativa-Lotus uliginosus						2			2
Trifolium pratense-Lotus uliginosus							3		3
Medicago sativa-Melilotus indicus							1		1
Rubus contrictus-Echium vulgare								1	1
Mixtas									
Retanilla trinervia-Persea americana	1								1
Eucalyptus globulus-Adesmia arborea		1							1
Quillaja saponaria-Lotus uliginosus			1						1
Quillaja saponaria-Brassica rapa			1						1
Quillaja saponaria-Medicago sativa				1					1
Quillaja saponaria-Rubus ulmifolius					1				1
Amomyrtus luma-Lotus uliginosus							1		1
Eucryphia cordifolia-Echium vulgare								2	2

El quillay, además de producir mieles monoflorales, fue importante en la producción de mieles biflorales mixtas desde la Región Metropolitana hasta la VII Región, ya que 4 de las 9 muestras de miel tipificadas en este trabajo como biflorales, presentaron significativamente, en su fracción polínica, polen de esta especie. En las mieles biflorales no nativas, la alfalfa (*M. sativa*) fue la especie más frecuente, con una participación de un 50% de las muestras analizadas, centrándose su producción en la VIII y IX Región (Cuadro 5).

Las mieles poliflorales se produjeron en todas las regiones estudiadas y correspondieron al 60% del total de muestras analizadas.

Los granos de polen de diez especies introducidas y 17 especies nativas se encontraron en porcentajes variables y estadísticamente significativos, en la fracción polínica de las muestras de mieles poliflorales no nativas y nativas, respectivamente (Cuadro 6).

Las especies con mayor frecuencia en la fracción polínica de las muestras de mieles poliflorales no nativas fueron lotera (*L. uliginosus*) y zarzamora (*R. ulmifolius*, Cuadro 6). La murra (*R. contrictus*) que reemplaza a la zarzamora, como maleza común en los bordes de los caminos y cercos, a partir de la IX Región, presentó una frecuencia de uso mucho menor que la zarzamora en la zona central. Esto probablemente se debió a una mayor cantidad y calidad del néctar ofrecido por especies forrajeras como el trébol rosado (*Trifolium pratense*) y lotera, que fueron las especies más frecuentes en las mieles poliflorales no nativas de la IX y X regiones.

Trece especies fueron importantes fuentes de néctar para las mieles poliflorales nativas producidas entre la IV y la VII regiones de Chile, mientras que sólo siete especies lo fueron en la IX y X regiones. Se puede distinguir de esta manera, una Zona Central, cuyas mieles presentaron la mayor diversidad específica en su origen botánico y una Zona Sur con una diversidad reducida (Cuadro 6). Esto se relaciona bien con el hecho que la Zona Central

Cuadro 6. Especies utilizadas por *Apis mellifera* como fuente de néctar en mieles poliflorales no nativas y nativas.
Table 6*. Species used by* Apis mellifera *as source of nectar in multifloral honeys, non native and native botanical origin.*

Especie	Número (no.) de muestras de miel por regiones administrativas de Chile[1]								Total no.
	IV	V	RM	VI	VII	VIII	IX	X	
Mieles poliflorales no nativas									
Eucalipto (*Eucalyptus globulus*)	4	1	2	9	4		6	1	27
Yuyo (*Brassica rapa*)	4	2	5	1	1		1		14
Lotera (*Lotus uliginosus*)	2		3	10	3	7	13	3	41
Trevul (*Melilotus indicus*)	1	1		1			7		10
Zarzamora (*Rubus ulmifolius*)	1	4	2	10	8	12			37
Hierba dulce (*Echium vulgare*)			2	1	2	17	7	3	32
Alfalfa (*Medicago sativa*)			4	6	1	13	1		25
Trébol rosado (*Trifolium pratense*)				1			13	1	15
Trébol blanco (*Trifolium repens*)							1		1
Murra (*Rubus contrictus*)							3	2	5
Mieles poliflorales nativas									
Tevo (*Retanilla trinervia*)	1	2	3						6
Molle (*Schinus latifolius*)	4	1							5
Heliotropo (*Heliotropium stenophyllum*)	1								1
Quillay (*Quillaja saponaria*)	1	1	7	5	3				17
Corontillo (*Escallonia pulverulenta*)	2		3	2	2			1	10
Litre (*Lithrea caustica*)		2	1		2				5
Boldo (*Peumus boldus*)		2							2
Quebracho (*Senna cumingii*)		1							1
Bollén (*Kageneckia oblonga*)		1							1
Arrayán (*Luma apiculata*)			2	3	3		1	3	12
Maqui (*Aristotelia chilensis*)			2		1		2		5
Culén (*Otholobium glandulosum*)				2					2
Tola blanca (*Proustia pyrifolia*)				1					1
Ulmo (*Eucryphia cordifolia*)							5	5	10
Tineo (*Weinmannia trichosperma*)							3		3
Tiaca (*Caldcluvia paniculata*)								1	1
Avellano (*Gevuina avellana*)								1	1

[1] Número de muestras de miel por regiones en las cuales el polen se encontró en proporciones estadísticamente significativos.
[1] Number of samples by region and the species found in statistically significant percentage.

de Chile presenta la mayor diversidad florística del país, la cual va disminuyendo hacia las zonas más meridionales. El arrayán (*Luma apiculata*) y el maqui (*Aristotelia chilensis*) fueron especies que aportaron néctar en ambas áreas, lo que se podría relacionar con su distribución geográfica más amplia y con cierta preferencia de *A. mellifera* por el néctar de estas especies.

En mieles poliflorales mixtas, las especies nativas más frecuentemente representadas en la fracción polínica fueron *Q. saponaria* y *L. apiculata* y las especies introducidas fueron *L. uliginosus* y *R. ulmifolius*. Además, se encontró otras ocho especies nativas y siete especies introducidas, en combinaciones, proporciones y frecuencias diversas, determinando en su conjunto este tipo de miel, lo cual revela la importancia de estas especies en la producción apícola-chilena (Cuadro 7).

Si bien el corontillo (*E. pulverulenta*) y el quillay (*Q. saponaria*), especies endémicas de Chile, además del ulmo (*E. cordifolia*), el avellano (*G. avellana*) y el tineo (*W. trichosperma*), especies nativas de Chile y Argentina, fueron las únicas que generaron mieles monoflorales endémicas y nativas, respectivamente, las tres primeras especies tuvieron una participación importante en la generación de mieles biflorales y poliflorales nativas y mixtas. Estos resultados avalan la importancia de las especies antes mencionadas en la producción apícola nacional. Las mieles monoflorales presentan

Cuadro 7. Especies utilizadas por *Apis mellifera* como fuente de néctar en mieles poliflorales mixtas.
Table 7*. Species used by* Apis mellifera *as source of nectar in mixed multifloral honeys.*

Mieles poliflorales mixtas	Número (no.) de muestras de miel por regiones administrativas de Chile[1]								Total no.
	IV	V	RM	VI	VII	VIII	IX	X	
Especies nativas									
Quebracho (*Senna cumingii*)	1								1
Quillay (*Quillaja saponaria*)	1		4	2	2				9
Corontillo (*Escallonia pulverulenta*)		1	2		2				5
Molle (*Schinus latifolius*)		1							1
Arrayán (*Luma apiculata*)		1		1	1	2	3		8
Tevo (*Retanilla trinervia*)			1	1					2
Maqui (*Aristotelia chilensis*)				1	1	2			4
Avellano (*Gevuina avellana*)						1			1
Lilén (*Azara petiolaris*)							1		1
Ulmo (*Eucryphia cordifolia*)							1		1
Especies introducidas									
Yuyo (*Brassica rapa*)	2	1	2			1			6
Eucalipto (*Eucalyptus globulus*)	1		1	1					3
Trevul (*Melilotus indicus*)	1						1		2
Rábano (*Raphanus sativus*)		1							1
Alfalfa (*Medicago sativa*)		3	1		1				5
Zarzamora (*Rubus ulmifolius*)		1	2	2	3	2			10
Lotera (*Lotus uliginosus*)			3	2			4		9
Hierba dulce (*Echium vulgare*)					1	2			3
Trébol rosado (*Trifolium pratense*)							2		2

[1] Número de muestras de miel por región en las cuales el polen se encontró en porcentajes estadísticamente significativos.
[1] *Number of samples by region and the species found in statistically significant percentage.*

grandes posibilidades para transformarse en productos con denominación de origen, debido al endemismo nacional o regional que presentan las especies que las originan.

La producción de mieles monoflorales y poliflorales pudo segregarse en dos grandes áreas correspondientes a dos zonas geográficas distintas desde un punto de vista climático y vegetacional. La primera correspondió a la Zona Central de Chile (IV a VII regiones), con un clima mediterráneo y una vegetación nativa principalmente dominada por un matorral arbustivo esclerófilo. Aquí se produjeron mieles poliflorales nativas con la mayor diversidad específica en su origen botánico y dentro de esta área, pudo segregarse una subárea conformada por las regiones V y Metropolitana, las cuales formaron un centro de origen de mieles monoflorales endémicas de corontillo y quillay.

La segunda área correspondió a la Zona Centro-Sur de Chile (VIII a X regiones), con un clima transicional mediterráneo-templado y bosques templado lluviosos caducifolios y siempreverdes, con un alto endemismo a nivel regional. Esta área destacó como centro de origen de mieles monoflorales nativas, donde es importante resaltar la producción de miel de ulmo, principalmente en la X Región. Por otro lado, en la IX Región, se centró la producción de mieles monoflorales no nativas provenientes de especies pratenses utilizadas en pastura de animales o forraje.

Los resultados de este trabajo permitieron caracterizar la producción apícola chilena y definir áreas geográficas en función de los tipos de miel producidos. La producción se distingue por una gran variedad de tipos de mieles, con una alta participación de especies vegetales nativas, que le otorgan cualidades particulares, debido al alto endemismo de esta flora.

Se debe tener presente que el panorama revelado corresponde a un diagnóstico del potencial apícola chileno, el cual podrá variar

principalmente por razones relacionadas con el manejo productivo de los apiarios, el clima y la dinámica de secreción de néctar de las especies.

La metodología utilizada en esta investigación y estipulada en la norma chilena, corresponde a una técnica actualmente vigente y de uso común en este tipo de análisis (CODEX 2001; European Comission 2002). En los últimos años han surgido otras técnicas analíticas alternativas o complementarias, como espectroscopía de fluorescencia (Ruoff, 2006), espectroscopia raman (Goodacre et al. 2002), espectroscopia infrarrojo medio (*mid-infrared*) (Tewari *et al*. 2005), espectroscopia cercano a infrarrojo (*near-infrared*) (Davies 2002) y marcadores químicos como compuestos volátiles o compuestos fenólicos (Bogdanov *et al*. 2004; Tomas-Braberán 2001; Andrade *et al*. 1994). Estas técnicas presentan ventajas y desventajas con respecto al análisis melisopalinológico (Persano Oddo, 2004; Molan 1998), cuya principal deficiencia es la sub o sobre representación del polen de algunas especies en la miel y el nivel de experiencia que se requiere para llevar a cabo este análisis. Los marcadores químicos y técnicas infrarrojas son útiles para la distinción entre mieles monoflorales, pero resulta difícil la distinción entre mieles monoflorales y poliflorales, y la clasificación de estas últimas, ya que presentan perfiles físicos y químicos muy diversos (Ruoff, 2006). Por lo tanto, es necesario avanzar en la caracterización de la diversidad de mieles producidas en Chile y sus características, para poder intentar estas metodologías en las mieles chilenas y utilizarlas como medios complementarios para hacer más exacta y eficiente la determinación de su origen botánico.

Resumen

La producción apícola chilena se caracteriza por una gran variedad de tipos de mieles, con una alta participación de especies vegetales nativas, las que les otorgan cualidades particulares, debido al alto endemismo de su flora. Alrededor del 95% de la miel producida en Chile es exportada a granel, sin ningún valor agregado, lo que equivale al 1% de participación en el mercado de exportación melífera. Como es imposible tener alguna ingerencia en el comercio mundial de este producto, se hace necesario mejorar la competitividad diferenciando la producción y convirtiéndola en un producto particular, con características únicas. Un avance importante en este sentido ha sido el establecimiento de un sistema de trazabilidad y la implementación de la Norma Chilena Oficial (NCh2981.Of2005) de denominación de origen botánico mediante ensayo melisopalinológico. Para caracterizar botánica y geográficamente la producción de miel en Chile, se utilizó el método establecido por la norma, aplicándolo a 240 muestras de miel, representativas de la producción apícola del país, cosechadas en dos temporadas sucesivas. Los resultados permitieron identificar dos grandes áreas productoras: una con clima mediterráneo (Zona Central IV-VII regiones) donde se originaron mieles monoflorales endémicas y poliflorales nativas con alta diversidad específica en su origen botánico y otra con un clima de transición hacia el templado húmedo (Zona Centro-Sur VIII-X regiones) productora de mieles monoflorales nativas y no nativas y poliflorales nativas con un origen botánico de diversidad restringida.

Palabras clave: Abeja, *Apis mellifera*, miel, origen botánico, tipificación, mieles chilenas.

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Rapid Scanning of the Origin and Antioxidant Potential of Chilean Native Honey Through Infrared Spectroscopy and Chemometrics

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Abstract
Antioxidant compounds have the ability to trap free radicals; in honey, this capacity is related to the botanical origin of the sample, and therefore, there has been a growing interest in verifying the floral origin of beehive products and its relation with the polyphenolic compounds with potential antioxidant activity. A FTIR spectrum has been use to discriminate floral origin in Chilean monofloral samples and to predict their antioxidant capacity. Forty-nine honey samples from different geographical zones and botanical origin were classified according to melissopalynology analysis, and total phenolic and flavonoid contents were quantified by spectrophotometric methods. Discriminant analysis showed that Quillay (*Quillaja saponaria*), Corcolén (*Azara petiolaris*), and Tebo (*Retanilla trinervia*) honeys showed similarities related to their common geographical origin, while Ulmo (*Eucryphia cordifolia*) presents a differentiate behavior. The FTIR spectra were able to predict phenolic and flavonoid content, establishing the potential of spectroscopic tools for quality control in Chilean beehive industry.

Keywords Honeybee · PCA · Antioxidant · FTIR · Melissopalynology

Introduction

Antioxidant compounds in food have an important role as a health-protecting factor, and they reduce the risk for chronic diseases, but their main characteristic is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols, and flavonoids scavenge free radicals thus inhibiting the oxidative mechanisms that lead to degenerative diseases. Natural foods rich in compounds like vitamin C, vitamin E, carotenes, and phenolic acids have been preferred as a healthier choice.

Honey has been widely studied, specially his antioxidant, antimicrobial, and homeopathic activities (Ahmed et al. 2018; Alissandrakis et al. 2011; Bueno-Costa et al. 2016; Corbella and Cozzolino 2006; Machado De-Melo et al. 2018), since this product made from the nectar of flowers and plants inherits the natural occurring antioxidant compound present in the nectar. Studies about the properties and applications of honey have distinguished honey from different botanical origins, concluding a relation between the polyphenols and the antioxidant properties (Ciulu et al. 2016; Cornara et al. 2017; do Nascimento et al. 2018; Gašić et al. 2017).

In Chile, native plants used by bees as a nectar source result in the production of honey with particular characteristics. Approximately 95% of the honey produced in Chile is exported in bulk (Barrera and Valdés 2014). Nowadays, 18 different types of native monofloral honey have been identified in Chilean beehive industry, where *Quillaja saponaria* (Quillay) and *Eucryphia cordifolia* (Ulmo) honeys represent approximately 20% of production (Montenegro and Ortega 2013). The differentiation of several types of Chilean honey according to their particular biological origins could improve their competitive values on the international market; currently, between 7000 and 11,000 tons of honey are exported annually, especially to the European Union (Oficina de Estudios y Polìticas Agrarias 2017).

To classify the botanical origin of honey, a melissopalynology analysis can be used, but this method has proven to be complicated and tedious. Moreover, there is a need to assess the antioxidant properties of honey. Spectrophotometric methods are

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widely used to determine flavonoids and polyphenols in honey even though they give poor information about the composition of these compounds. Liquid chromatography and mass spectrometry have also been used, but unfortunately, these are more laborious and expensive methods in comparison with NIR, that is quick and require a minimal amount of sample and handling (Martínez del Río et al. 2013; Pascual-Maté et al. 2018).

An FTIR spectrum has the potential to bring information on compounds and their functional groups present in honey allowing to discriminate and authenticate different samples (Kasprzyk et al. 2018; Leme et al. 2018; Pascual-Maté et al. 2018). However, it is necessary to process the data using chemometric tools, such as main component analysis, partial least squares, and discrimination analysis to characterize and classify honey samples (Corvucci et al. 2015; Jandric et al. 2015; Popek et al. 2017; Song et al. 2016; Wen et al. 2017; Zhou et al. 2014).

The aim of this work is to apply FTIR analysis and chemometric tools to describe and differentiate two or more botanical origins in Chilean honey samples and predict antioxidant activities like total phenolic or flavonoid content in native honey.

Materials and Methods

Chemicals

Folin–Ciocalteu's phenol reagent, aluminum chloride, sodium chloride, hydrochloridric acid, disodium hydrogen phosphate dihydrate, ethanol, and sodium phosphate monobasic reagent were supplied by Merck (Darmstadt, Germany). Water was purified in a Milli-Q system (Synergy, Millipore, Darmstadt, Germany).

Honey Sampling

Honey samples were taken from local beekeepers from the O'Higgins (Mediterranean climate) and Araucanía (humid temperate climate) regions of Chile. A total of 49 samples were taken, including 20 *Quillaja saponaria* (Quillay), 14 *Eucryphia cordifolia* (Ulmo), 8 *Azara petiolaris* (Corcolén), and 7 *Retanilla trinervia* (Tebo) honey samples. They were stored in darkness and refrigerated at 4 °C until analysis.

Melissopalynology Analysis

The botanical/floral origin of the honeys was determined by a palynological analysis as described in the Chilean Normative for classification of honeybees (Nch2981 n.d. Of2005). To determine the botanical origin of the pollen in honey samples, specific literature (Heusser 1971; Marticorena and Quezada 1985) and a botanical palinotec library in Pontificia Universidad Católica de Chile were consulted. Unifloral honey types were those that come mainly from one species whose pollen composition was at least 45% of pollen from that plant. On the other hand, multifloral honey types were those made from the nectar of several species whose principal pollen did not reach a percentage equal to or greater than 45% and are considered bifloral honey types. These are determined by the fact that their composition is made significantly of pollen from two species (both ≥ 45%) and a similar proportion was found (with a difference of < 5%).

Determination of Total Flavonoids

Honey extracts were obtained based on the Montenegro patent (EP1852017-2010) and Ferreres et al. (1994) procedures. Honey samples were diluted in acidic water (pH 2 with HCl). Then, the dissolution was passed through an open chromatographic column filled with Amberlite XAD-2 and washed with acidic water, distilled water, and ethanol. Residues were concentrated with a vacuum rotary evaporator and redissolved in pure water. Finally, extracts were filtrated (EDLAB CA Syringe filter 0.45 μm) and stored at − 18 °C.

Total flavonoids were determined using Prelipcean et al. (2011) published procedures. Briefly, 1 mL of extract was mixed with aluminum chloride 2% in ethanol and let rest for 1 h at room temperature. Its absorbance was determined at 420 nm (Spectrophometer Agilent 8453; Software UV-Visible Chemstation Rev.A.10.01 Agilent Technologies 95-03). Calibration curve was prepared with quercetin, and the results were expressed in mg QE/100 g of honey.

Determination of Total Phenolic Compounds

Two hundred microliters of the previously described extract was taken and mixed with 50 μL of Folin-Ciocalteu Reagent, 150 μL of sodium carbonate solution (20% *w*/*w*), and 600 μL of ultrapure water. The solution was let to rest for 30 min at dark, and measure the absorbance of the samples at 765 nm. The calibration curve was prepared with gallic acid, and the results were expressed in mg GAE/100 g of honey.

IR Analysis

Honey samples were homogenized before IR analysis. A 200 mg of honey was deposited on KBr pellets of 0.1 mm optical path for their analysis on a Vector 22 spectrophotometer (Bruker Optics Inc., Germany) over the range of 250–4000 cm^{-1}. For each sample, 64 scans were recorded, with a spectral resolution of 2 nm.

Multivariate Analysis

FTIR spectra were centered and standardized before chemometric analysis. Matrix data was constructed by 49 × 1945 (49 samples and 1945 spectral point data). A principal component

Table 1 Melissopalynology analysis of four different botanical origins of Chilean honey samples

Sample	Primary species		Secondary species		Total phenolics content (mg GAE/100 g honey)	Total flavonoids content (mg QE/100 g honey)
	Scientific name	Percentage (%)	Scientific name	Percentage (%)		
Q1	*Quillaja saponaria* (Quillay)	46.6	*Galega officinalis*	19.0	13.2 ± 0.7	2.2 ± 0.2
Q2		68.5		25.0	12.1 ± 0.9	1.9 ± 0.1
Q3		50.9	*Crinodendron patagua*	12.7	14.2 ± 1.1	2.2 ± 0.1
Q4		47.8	*Trifolium repens*	21.7	10.5 ± 1.6	1.4 ± 0.2
Q5		76.3	*Medicago poymorpha*	8.3	12.3 ± 0.1	2.1 ± 0.1
Q6		49.6	*Galega officinalis*	32.3	12.7 ± 0.6	2.5 ± 0.2
Q7		48.2		24.5	8.4 ± 0.1	1.4 ± 0.2
Q8		46.0		17.0	13.3 ± 0.6	1.6 ± 0.5
Q9		62.2		15.2	8.1 ± 0.5	0.4 ± 0.1
Q10		38.9		20.1	12.9 ± 0.1	2.3 ± 0.1
Q11		70.7		15.9	11.7 ± 1.3	1.8 ± 0.4
Q12		40.4		21.2	12.9 ± 0.6	2.1 ± 0.1
Q13		43.9	*Azara* sp.	29.6	16.3 ± 0.1	2.6 ± 0.3
Q14		73.9	*Galega officinalis*	8.6	11.5 ± 1.1	2.5 ± 0.4
Q15		74.9		23.0	12.7 ± 0.6	1.9 ± 0.4
Q16		35.4		33.4	13.7 ± 0.4	2.5 ± 0.2
Q17		46.3		27.7	13.2 ± 0.7	1.9 ± 0.1
Q18		46.9		9.4	12.7 ± 0.4	2.5 ± 0.2
Q19		38.3		24.5	10.7 ± 0.6	1.8 ± 0.1
Q20		46.2		27.0	16.9 ± 0.7	2.7 ± 0.1
U1	*Eucryphia cordifolia* (Ulmo)	45.7	*Lotus pedunculatus*	19.4	180.4 ± 1.5	46.9 ± 1.5
U2		93.5		2.9	199.6 ± 6.7	42.7 ± 7.4
U3		89.1		7.7	185.5 ± 2.2	81.4 ± 3.7
U4		95.6	*Weinmannia trichosperma*	3.8	202.1 ± 4.4	82.3 ± 9.5
U5		89	*Luma apiculata*	6.2	184.6 ± 1.9	75.5 ± 4.3
U6		98		1.9	183.8 ± 2.1	78.4 ± 3.3
U7		97.2	*Weinmannia trichosperma*	2.8	188.6 ± 4.3	90.1 ± 6.9
U8		92.9	*Lotus pedunculatus*	2.6	176 ± 9.5	83.1 ± 5.9
U9		96		2.5	180.4 ± 4.1	70.5 ± 1.3
U10		94.9	*Weinmannia trichosperma*	3.5	181.6 ± 2.6	70.1 ± 2.6
U11		97.2	*Luma apiculata*	1.6	177.3 ± 8.4	83.3 ± 9.4
U12		68.4	*Weinmannia trichosperma*	22.9	180.6 ± 2.1	59.7 ± 0.5
U13		49.1	*Lotus pedunculatus*	41.4	178.4 ± 2.8	66.1 ± 2.6
U14		93.6	*Azara/Salix*	9.1	155.5 ± 2.4	54.1 ± 9.2
C1	*Azara petiolaris* (Corcolén)	51.7	*Galega officinalis*	15.9	66.3 ± 7.4	12.3 ± 0.9
C2		76.6		6.7	153.3 ± 6.1	11.9 ± 0.8
C3		45	*Luma/Myrceugenia*	33	72.9 ± 2.5	8.3 ± 0.6
C4		43.4		39	65.1 ± 2.3	8.7 ± 0.6
C5		58.5	*Brassica* sp.	20.2	48.8 ± 1.1	5.9 ± 0.4
C6		63.9	*Escallonia* sp.	11	128.4 ± 4.4	13.6 ± 0.9
C7		83.6	*Luma/Myrceugenia*	8	135.2 ± 4.3	38.3 ± 3.1
C8		73		7.1	126.2 ± 2.9	10.9 ± 0.7
T1	*Retanilla trinervia* (Tebo)	77.96	*Robinia pseudoacacia*	11.84	157.5 ± 6.6	104.8 ± 1.5
T2		63.14	*Talguenea quinquinervia*	11.3	194.1 ± 1.2	127.1 ± 4.6
T3		45.32	*Myrceugenia*	9.98	204.2 ± 6.5	84.8 ± 1.3
T4		47.82	*Brassica* sp.	13.4	154.2 ± 1.6	56.6 ± 5.5
T5		47.96	*Galega officinalis*	11.1	250.3 ± 8.9	108.6 ± 2.4

Table 1 (continued)

Sample	Primary species		Secondary species		Total phenolics content (mg GAE/100 g honey)	Total flavonoids content (mg QE/100 g honey)
	Scientific name	Percentage (%)	Scientific name	Percentage (%)		
T6		56.15	*Salix* sp.	11.3	179.5 ± 4.2	98.2 ± 5.7
T7		56.23	*Crinodendron patagua*	24.36	178.5 ± 2.2	105.1 ± 4.1

analysis (PCA) was performed on the data to obtain an overall multidimensional view. The number of components required to explain the data adequately was selected by cross-validation.

The matrix of scores generated in the PCA, consisting of 49 rows (samples) and *A* columns, where *A* = number of components extracted by cross-validation, was used to build a classification model using linear discriminant analysis (LDA). The A initial PCs were further reduced by backward elimination on the basis of their partial *F* values in the discriminant models.

A prediction model of total content of polyphenol and flavonoids was created using partial least square method (PLS1). The X-variable was spectra data, and Y-variable was the total flavonoids and polyphenols concentrations. The optimum number of factors to be used within the PLS regression was determined through a full cross-validation procedure, which consisted of systematically removing one of the training samples, and using only the remaining ones for construction of the latent factors and/or regression coefficients. All data was previously centered and standardized.

PCA and PLS1 were built using The Unscrambler v9.7 CAMO software, while Statgraphics Centurion XV software was used for LDA.

Results and Discussion

Classification by Linear Discriminant Analysis

The melissopalynology analysis results are listed in Table 1. Most of the samples were cataloged as monofloral honeys accordingly with the Chilean normative (Montenegro et al. 2008). However, a wide range of secondary species have been observed, especially in honey from the central zone where diverse flora grows. In Quillay honey, the most abundant secondary species is G*alega officinalis* with high percentages up to 33%. Ulmo honey showed *Lotus pedunculatus* as its most abundant secondary species; but in all samples, the other species represent less than the 22% of the contribution. Corcolén and Tebo honeys do not have a characteristic secondary species, representing less than 32% of the botanical origin. Important to highlight is the contribution of introduced species such as *Galega officinalis* (galega), *Trifolium repens* (white clover), and *Brassica* sp. (cabbage, cauliflower, broccoli, brussel sprouts) in samples from Central Chile, a reflection of its agricultural industry. On the other hand, low percentages of endemic species of Chile like *Weinmannia trichosperma* (Tineo) and *Lotus pedunculatos* (*Alfalfa chilota*) were observed in samples from southern Chile.

Total phenolic content and total flavonoids content shown in Table 1 have been used as preliminary screening of antioxidant capacity, since they are widely use in food matrices (Bridi et al. 2015). A significantly lower content of phenolics was observed in Quillay honeys when compared with the other honey samples in this study, and with some international monofloral honeys such as the famous Manuka honey (*Leptospermum scoparium*) or the black forest honey (*Acacia* sp.), with a total polyphenol value reported of 89.9 ± 1.2 mg and 62.7 ± 4.4 GAE/100 g of honey, respectively (Alzahrani et al. 2012).

In accordance with the FC results, Quillay honey presents a lower flavonoid content than the other samples. However, a previous reported study with 26 Chilean honey samples establishes that flavonoid content can vary from 0.014 to 13.80 mg QE/100 g of honey (Muñoz et al. 2007).

IR Honey Samples

Samples were analyzed by IR since it is a relatively easy and quick technique to address honey composition. The fingerprint spectra of a representative sample for each honey type (Quillay, Corcolén, Tebo, and Ulmo) are shown in Fig. 1.

The four types of honeybees have a remarkable band related to water signal at 3600–3000 cm^{-1}, associated to the OH stretching, since water along with fructose and glucose represents 95% of honey composition. The signal at 2900–2800 cm^{-1} should correspond to the C–H stretching of the methyl and methylene groups of the sugar molecules. Also, in all the samples, a peak at 1700–1600 cm^{-1} corresponds to the C=O stretching absorption of aldehyde group from glucose and fructose compounds. As expected, all samples showed a peak at 1450–1400 cm^{-1} assigned to the antisymmetric deformation of methyl and the scissors vibration of methylene groups and a defined 1200–1050 cm^{-1} signal assigned to the stretching vibration absorption of C–OH from typical alcohol and phenol structures (Svečnja et al. 2015).

However, relevant difference can be observed in the fingerprint zone. Quillay, Corcolén, and Tebo honey types showed similar signal in the 2500–2200 and 1800–1500 cm^{-1} spectra regions due to C–H stretching and aliphatic C=O stretching,

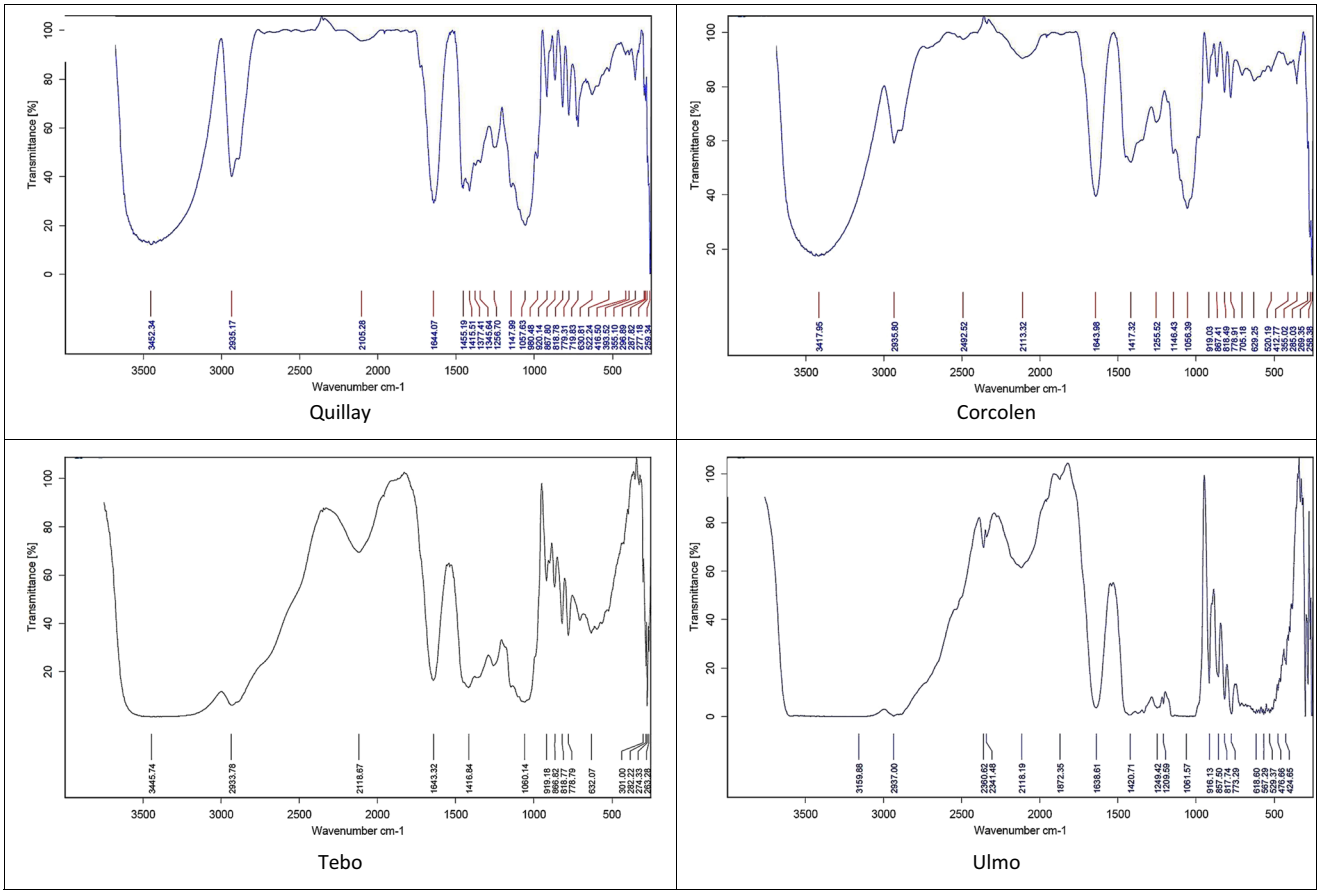

Fig. 1 Representative IR spectra from different botanical origins of four native Chilean honey types

respectively, which may be linked to their common geographical origin (Region of O'Higgins). Ulmo honey shows a more distinctive signal a 1750–1710 corresponding to the stretching vibration absorption of C=O carbonyl group. On the other hand, Ulmo's honey showed saturation in the 900–500 and 1800–1600 cm^{-1} regions, which is characteristic of methylic and phenolic compounds and cetonic groups, respectively. Thi slightly different could indicate that the composition of honey, reflected in the IR spectra, could be related to both its botanical and geographical origin.

Principal Component Analysis

The IR spectra of the samples in the range 4000–400 cm^{-1} were analyzed by PCA, a common statistical tool to analyze data from complex matrices such as honey. Figure 2 shows the score plot for the first two main components that explains 79 and 13% of the variance of data, respectively (both PCs explain 92% variance of data). As it is evident, Ulmo honey forms a distinct group from the rest of the samples. Honey from Quillay and Tebo is distinguishable from one another, yet Corcolén honey appears in both groups; this could indicate that the difference in composition given by botanical origin is less significant than the one given by the geographical origin. Thus, honey from Quillay, Tebo, and Corcolén collected in the same region showed similar IR spectra and appeared to be less differentiated from one another using only this information.

Botanical Origin

In LDA, eight initial PCs were further reduced to seven by backward elimination on the basis of their partial *F* values in the discriminant model. The incorporation of more than seven PCs makes greater misclassification probable since they are only modeling the noise of IR spectra.

Table 2 shows the classification honey by LDA. For Ulmo and Tebo honey types, all samples were correctly classified (7 Tebo and 14 Ulmo honey samples), while for Quillay and Corcolén honey, the classification rate was 80% and 75%, respectively. This misclassification could have been produced by the similarity in the composition of the nectar source due to weather conditions but also could be important to consider that those honey types share botanical composition due to the presence of *Galega officinalis* and *Luma apiculata* as secondary species in most of the samples.

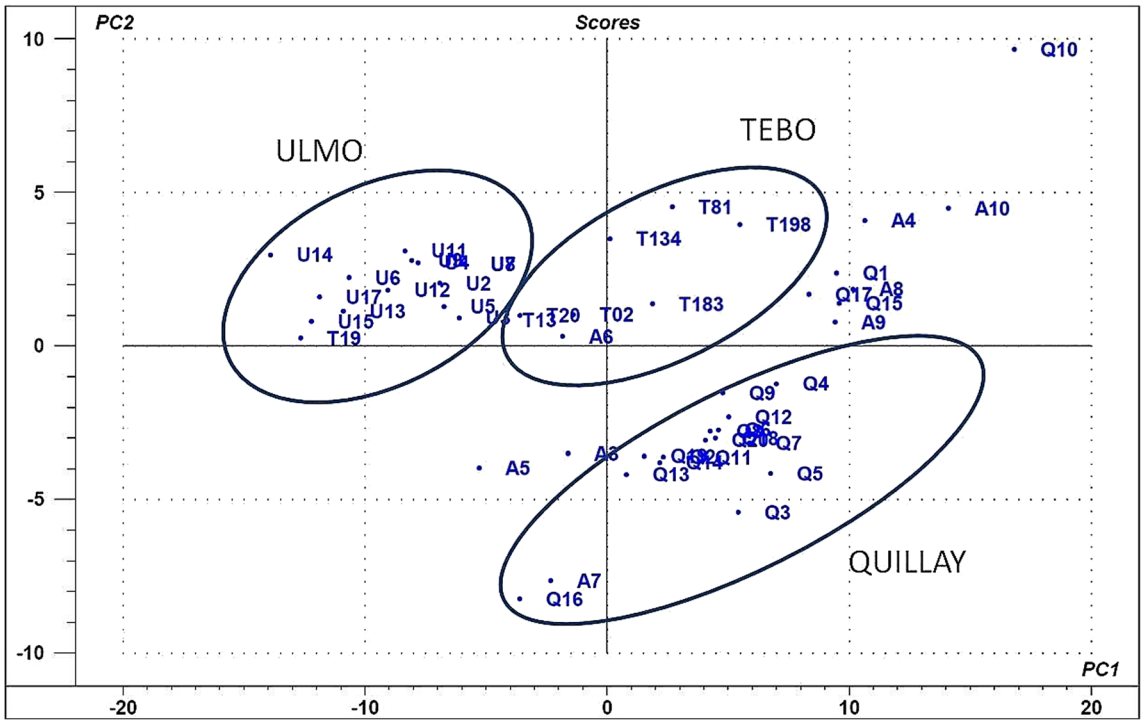

Fig. 2 Score plot of PCA at IR spectra Chilean honey

Geographical Origin

Honey samples were taken from two different Regions with different climate. The central zone of the country has Mediterranean climate and hence helps produce honey from endemic unifloral and native multifloral botanical origins, while the south area of the country is characterized by a humid temperate climate and thus produces both native and non-native unifloral and native multifloral varieties with restricted botanical diversity (Montenegro et al. 2013).

In this regard, vegetation in those regions is diverse and does not necessarily share the same native and endemic species. IR analysis gives a correlation between functional group and concentration of them in honey. There is a good correlation between IR honey spectra and their geographical origin (Fig. 3).

In predicted geographical origin by PLS1 model, regression data showed a good correlation (R-Square near to 0.9): Ulmo honeys can be easily differentiated from the other three honey types, showing that samples from O'Higgins region share similar characteristics, having similar data range (a little compacted).

Prediction of the Antioxidant Potential of Honey

A PLS regression was carried out between IR spectra (X variable) and total phenolic or total flavonoid content (Y variable) for all honey samples. The score plots and the predicted concentrations compared with the measured values obtained experimentally are displayed in Fig. 4. For these models, four and three PLS factors were selected by cross-validation that accounts for the variance in phenolic and flavonoid content. A significant correlation was observed between predicted and measured values for both parameters (R-square = 0.82 and 0.73 in cross-validation, respectively). Conversely, the values of slope obtained were lower than 1.00 (0.84 and 0.77 for polyphenols and flavonoid) in both cases, indicating an underestimation in its content by the IR-PLS1 model.

Table 2 Prediction of classification honeys by discriminant analysis

Botanical origin	Predicted botanical origin				
	Corcolén	Quillay	Tebo	Ulmo	% correct classification
Corcolén ($n = 8$)	6	2	0	0	75
Quillay ($n = 20$)	4	16	0	0	80
Tebo ($n = 7$)	0	0	7	0	100
Ulmo ($n = 14$)	0	0	0	14	100

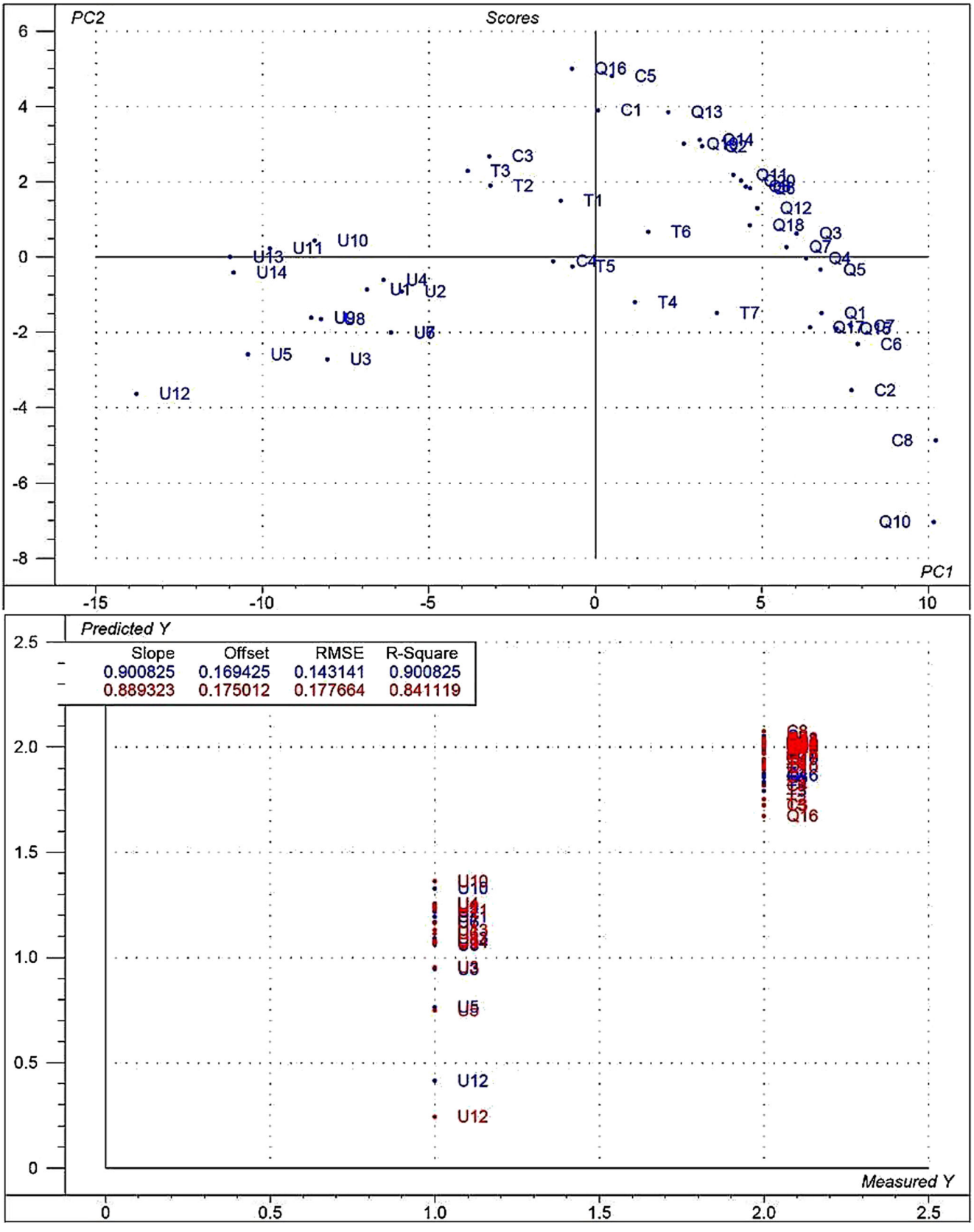

Fig. 3 Score plot and predicted data of geographical-IR spectra correlation by PLS1 model

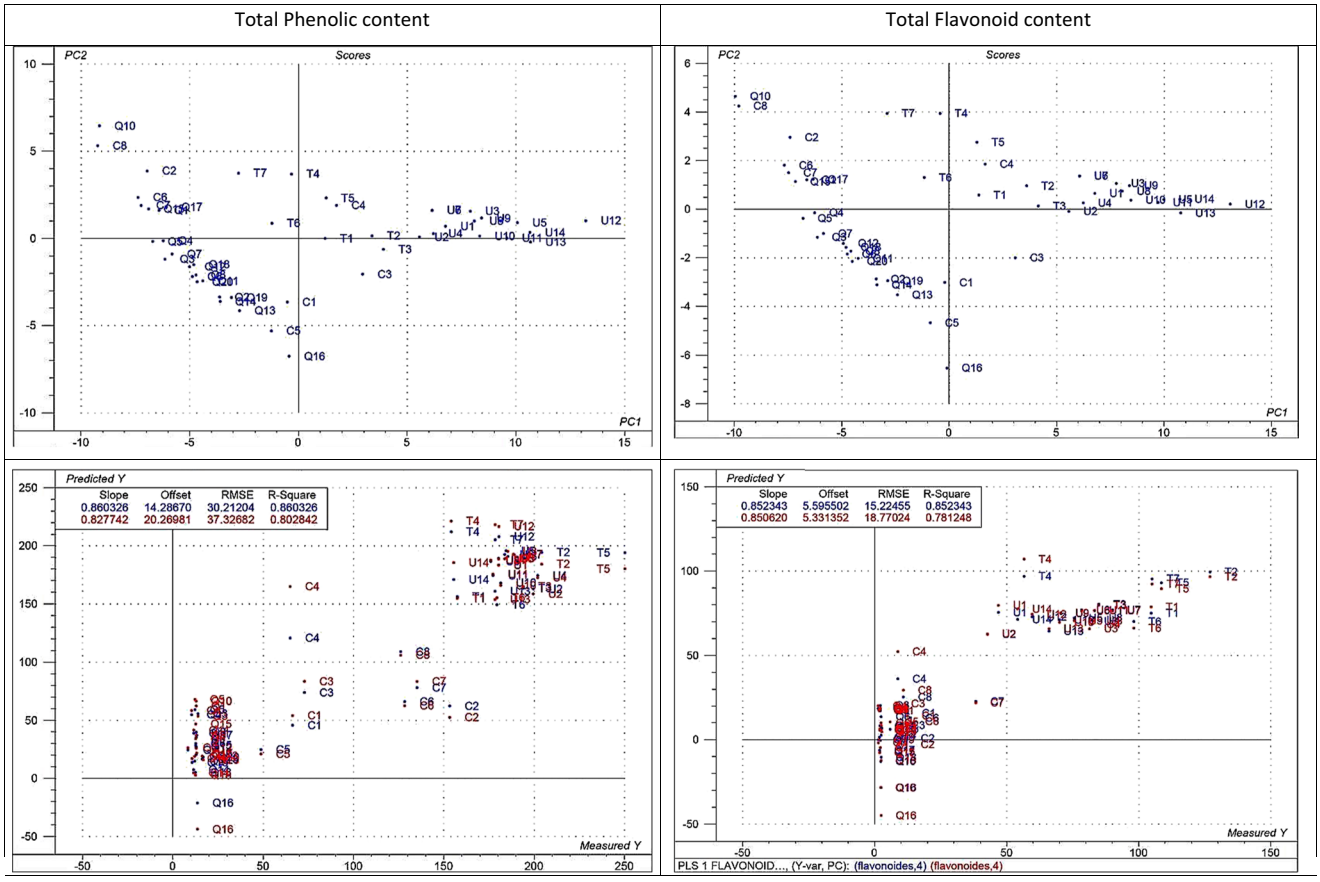

Fig. 4 Prediction of total phenolic and flavonoid contents in Chilean honey types by PLS1 model

However, as seen in Fig. 4, the concentration of both parameters was predicted incorrectly in Quillay honey samples. This honey type had the lowest concentration of polyphenol and flavonoid content (lower than 20 mg GAE or QE/100 g of honey) that could imply that the PLS models are appropriate for honey samples with higher values of flavonoid and phenolic content, as was determined in Ulmo, Corcolén, and Tebo honey samples. RMSE value of PLS model was 30 mg GAE/100 g honey and 15 mg QE/100 g honey for polyphenol and flavonoid content, respectively, and RMSE represents a variance lower than 20% of values founded in Ulmo, Corcolén, and Tebo. These values were actually predicted by the PLS models with some level of dispersion related to the spectral variability of the samples, and considering that both FC method and Prelipcean method values can be overcalculated due to interference such as sugar residues in the extracts (Bridi et al. 2015), suggesting that the error in prediction could be linked to error in the experimental analysis.

Conclusion

IR spectra analysis on Chilean honey samples can offer very important data such as botanical origin or antioxidant activity when chemometric data analysis is applied. Prediction of important properties of honey like botanical origin and total phenolic and flavonoid compounds offers important information allowing us to be aware of the quality of Chilean honey samples in a short time. The PLS models can be used as a quick review of honey samples by NIR to establish antioxidant potential, providing an estimate of the contents of polyphenols and flavonoids. This approach also could be used as a tool to identify unifloral honeys or adulteration in samples.

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Compliance with Ethical Standards

Conflict of Interest Ady Giordano declares that she has no conflict of interest. Mauricio Retamal declares that he has no conflict of interest. Edwar Fuentes declares that he has no conflict of interest. Loreto Ascar declares that he has no conflict of interest. Patricia Velásquez declares that

she has no conflict of interest. Karina Rodríguez declares that she has no conflict of interest. Gloria Montenegro declares that she has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

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Chapter 3

The Value of Chilean Honey: Floral Origin Related to their Antioxidant and Antibacterial activities

Raquel Bridi and Gloria Montenegro

Additional information is available at the end of the chapter

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Abstract

Honey chemical composition is related to the plant species where nectar is collected by honeybees. Chilean beekeeping is characterized by a variety of honey types, some unique, due to a high participation of endemic and native species. In Chile, the most emblematic flower honey, both for its abundance and sensory characteristics, is ulmo honey (*Eucriphya cordifolia*) and quillay honey (*Quillaja saponaria*). Melissopalynological analyses are used to establish whether a honey is unifloral, where at least 45% or more pollen grains found in it belong to the same species. The antioxidant and antimicrobial activities of Chilean honey have been studied in the last years with excellent results. *Quillaja saponaria, Eucriphya cordifolia, Azara petiolaris*, and *Retanilla trinervia* are within the Chilean endemic species that produce unifloral honeys that show antioxidant potential and antibacterial activity against pathogenic gram positive and gram -negative bacteria and also multiresistant strains. These activities are mainly attributed to the phenolic compounds such as flavonoids. Among these attractive characteristics of honey, it is important to note that this product has low toxicity and the medicinal properties of honey will help to protect honeybees by adding value not only to the significantly important process of pollinating crops and native plants, but also for the medicinal importance of their products.

Keywords: Chile, *Apis mellifera*, honey, phenolic compounds, biological activity, antioxidant, antibacterial

1. Introduction

In Chile, the natural and endemic flora offers many plants with invaluable potential biological properties that may be inherited for products originated from this flora such as honeybee products. Likewise, beekeeping is active and there are several unifloral and endemic Chilean honey that have been reported to have important biological properties such as ulmo

honey (originating from *Eucriphya cordifolia*), quillay honey (originating from *Quillaja saponaria*), tevo honey (originating from *Retanilla trinervis*), and others. The botanical origin of honey may be known through a quantitative and qualitative melissopalynological analysis. Honeybees are selective in the use of flower resources. The dominant plant community in Central Chile corresponds to the Matorral, an evergreen sclerophyllous vegetation with quillay and tebo as dominant plant species. The deep south of the country is dominated by temperate forest where ulmo is one of the dominant species. Biodiversity varies along an altitudinal or latitudinal gradient in Chile, so the beekeepers usually maintain their beehives along the native plant communities, so the bee products, as well as their potential biological properties will also be different depending on the botanical and geographical origin. Among the bioactive molecules inherited from a specific floral source, phenolic compounds obtained from honey have been related with the antioxidant and antibacterial properties that they show. Honey as a natural product offers many advantages that classify it as an excellent source of active molecules, which could be used as a treatment of human diseases in the forthcoming years. Among these attractive characteristics of honey, it is important to note that this product has low toxicity and the medicinal properties of honey will help to protect honeybees from disappearance by adding value not only to the significantly important process of pollinating crops and native plants, but also for the medicinal importance of their products.

2. Chile's unique geographical features and its endemic flora

The continental Chilean territory has an area close to 75 million hectares and is situated on the southwest border of South America. It has a length of approximately 4300 km from north to south and the average width is 180 km. Pits, terraces, mountainous regions, and valleys form Chile's diverse geomorphology, which together with the biogeographical isolation of a territory limited by geographical and climatic barriers, has configured a biodiversity characterized by a high level of endemism in ecosystems. Chile's vascular flora contains approximately between 5500 and 6000 species, without including subspecies and varieties. Although the number of species, compared with other South American countries, is not especially high, the most prominent trait of Chilean vascular flora is the presence of close to 50% endemic plant species, which gives the Chilean vascular flora a marked uniqueness [1]. Chilean apicultural production is defined by a high variety of honey types which contain a high percentage of nectar obtained from native plant species. The portion of nectar originating from native plants related to the endemism of Chilean flora result in the production of honey with unique characteristics.

The production of native and endemic monofloral honey is segregated into two large geographical areas: the first area corresponds to the central zone of Chile and the second corresponds to a region with a climatic transition from humid Mediterranean (VIII Region) to temperate humid (X Region). The central zone of Chile is of the five regions in the world that has a Mediterranean climate. It is characterized by a high level of endemism and biodiversity. Matorral is the dominant vegetal community in this zone. Characteristic matorral species include *Baccharis concave* (chilca), *Peumus boldus* (boldo), *Lithraea caustica* (litre),

http://dx.doi.org/10.5772/67103

Trevoa trinervis (tevo), and *Q. saponaria* (soapbark tree). The central zone is characterized by the production of endemic monofloral honey from the quillay (*Q. saponaria*) and corontillo (*Escallonia pulverulenta*) species, while the southern zone, characterized by temperate forests, is characterized by native unifloral honey made from avellano *(Gevuina avellana)*, ulmo *(E. cordifolia)*, and tineo *(Weinmannia trichosperma)* [2].

3. Botanical origin of honey

Honeybees show great selectivity in the use of the vegetation surrounding their beehives. It has been shown that bees select plants with a high production of nectar, high concentration of sugar and that do not contain toxic compounds like certain alkaloids. Nevertheless, the presence of other secondary metabolites including terpenoids, phenolic acids, and flavonoids confer to honey import medicinal properties [3]. Nectar is an aqueous plant secretion whose content is mainly sugars and amino acids. It is collected by bees, particularly *Apis mellifera* L., and is converted into honey by enzymatic actions and dehydration, producing about 18% water content [4]. Honey is a food that contains about 200 substances and consists mainly of sugars, water, and other substances such as proteins (enzymes), organic acids, vitamins (especially vitamin B6, thiamine, niacin, riboflavin, and pantothenic acid), minerals (including calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), pigments, solid particles derived from honey harvesting, a large variety of volatile compounds, and also secondary metabolites characteristic of the origin species like phenolic compounds and terpenes [4, 5].

Melissopalynology is the division of palynology, which studies the botanical and geographical origin of honey by subjecting honey sediment, and therefore pollen grain and the other structures therein, to microscopic analysis (**Figure 1**). Honey pollen profiles indicate floral diversity, forest vegetation, and species composition of plants that honeybees forage. The relative pollen frequency is utilized for tagging purposes and to ensure geographical origin, factors which considerably influence honey's commercial value. Furthermore, relative pollen frequency is also utilized as a traceability tool by food control institutions and to assess correlations with *in situ* climatic parameters such as rainfall and temperature, important external factors influencing pollinators and pollination networks [6, 7]. In Chile, the official policy (NCh2981.Of2005) established by the Standards Division of the National Institute for Standardization [2] indicates that the melissopalynological test must be used to differentiate the botanical origin of honey produced in this country. In agreement with this regulation, honey can be classified according to three types of botanical origins: monofloral, bifloral, or polyfloral. Monofloral or unifloral honeys are those where at least 45% or more pollen grains found in it belong to the same species; bifloral honeys are those where pollens from two species are dominant within the total pollen grains, so that, as a whole, both species cover more than 50% of the total pollen grains, and there is not a difference higher than 5% among them; and finally, polyfloral honeys are those where none of the requirements for monofloral and bifloral honeys are met, that is, those where no species reaches at least 45% of the total pollen grains, nor two of them covers more than 50% of the said total.

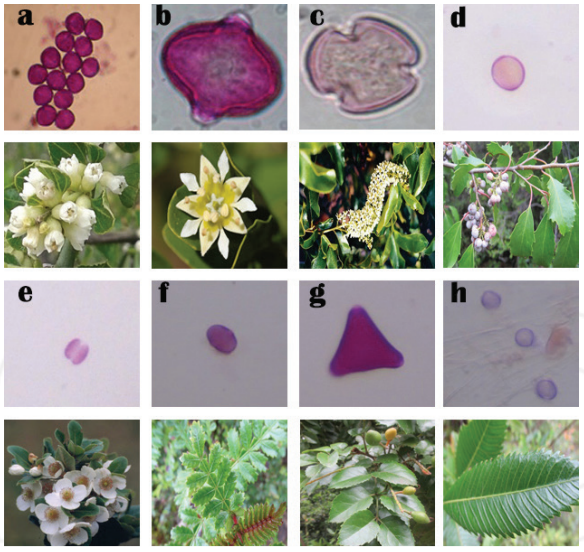

Figure 1. Plants species and respective pollens (microscope 400x) (a) *Retanilla trinervia* (tevo), (b) *Quillaja saponaria* (quillay), (c) *Escallonia pulverulenta* (corontillo), (d) *Azara petiolaris* (corcolén), (e) *Eucryphia cordifolia* (ulmo), (f) *Weinmannia trichosperma* (tineo), (g) *Gevuina avellana* (avellano chileno), and (h) *Caldcluvia paniculata* (tiaca).

The melissopalynology technique is quite laborious, time-consuming and requires a high-skilled and trained technician. Thus, a large number of research groups worldwide have focused their attention and studies on improving the knowledge of honey characterization. The most promising approach appears to be the simultaneous detection of multiple components utilizing spectroscopic methods along with statistical analysis. Chemometrics along with Raman, FTIR, NMR, and NIR spectroscopic methods have been used for defining the floral origin of honey and development of classification models. These procedures promptly provide quantitative information without complex pretreatment of samples and primarily through a single spectroscopic technique [6, 8, 9]. Despite advances in these methods in the last few years, limitations still exist in these studies related to the small number of samples and the validity of the proposed methods are rarely demonstrated [8]. Notwithstanding, the emerging new methods are making way to new frontiers in honey characterization. The most promising strategy appears to be the multidisciplinary one, which focuses on the detection of multiple components assisted by chemometrics. Apicultural industries and small producers will make the most of the advantages of more advanced methods which allow for more scrupulous controls, increasing the quality level and safety of honey and derivatives [9].

4. Chilean unifloral honey

Chile produces a limited number of unifloral honeys with native plant origins. Montenegro et al. [10] identified the species of native plants that *A. mellifera* uses as the most intensive

http://dx.doi.org/10.5772/67103

source of nectar. These species include *Q. saponaria* (quillay, soapbark), *E. cordifolia* (ulmo), *G. avellana* (avellano), *E. pulverulenta* (corontillo), *R. trinervia* (tevo), *Caldcluvia paniculata* (tiaca), *W. trichosperma* (tineo), and species of genus *Azara* (corcólen). They are used as the source for monofloral honey, which are selected by honeybees mainly due to the volume and chemical composition of nectar offered by the flowers [4, 7, 10] (**Table 1**).

Origin	Common name	Plant species	Family
Zone			
Forest temperate (Southern Chile)	ulmo	*Eucryphia cordifolia* Cav.	Cunoniaceae
	tiaca	*Caldcluvia paniculata* (Cav.) D. Don	Cunoniaceae
	tineo	*Weinmannia trichosperma* Cav.	Cunoniaceae
	avellano	*Gevuina avellana* Molina	Proteaceae
Matorral (Central Chile)	quillay	*Quillaja saponaria* Monlina	Quillajaceae
	tevo	*Retanilla trinervia* (Gillies & Hook.) Hook. & Arn.	Rhamnaceae
	corontillo	*Escallonia pulverulenta* (Ruiz & Pav.) Pers.	Escalloniaceae
	corcolen	*Azara petiolaris* (D. Don) I.M. Johnst.	Salicaceae

Table 1. Botanical origin of unifloral honey in Chile.

A recent study of the biological properties of Chilean unifloral honeys indicates that Chilean native honey presented significant differences in their antioxidant as well as biological activity, which depends on the botanical and geographical origin, and can be associated with polyphenol content. Moreover, the presence of other species in the total botanical content of honey plays an important role in the modulation of its biological properties [11].

In Chile, the most emblematic flower honey, both for its abundance and sensory characteristics, is quillay (*Q. saponaria)* and ulmo (*E. cordifolia*). The antioxidant and antimicrobial activities of Chilean honey have been studied in the last years with excellent results. *Q. saponaria*, *E. cordifolia*, and *R. trinervia* are within the Chilean endemic species that produce monofloral honey that show antibacterial activity against pathogenic Gram-positive and Gram-negative bacteria and also multiresistant strains [4, 10–14]. With regard to antioxidant activity, honey from *Q. saponaria* and *Azara petiolaris* stand out due to potential shown in various *in vitro* models utilized to evaluate natural antioxidant capacity to inactivate reactive species. The positive correlation between phenolic compounds and antioxidant capacity is verified in some of these models. Phenolic compounds such as aromatic acids and flavonoids are considered to be responsible for antioxidant capacity since they have a chemical structure particularly suitable to exert an antioxidant action acting as free radical scavengers neutralizing reactive oxygen species and chelating metal ions.

5. Phenolic compounds

Phenolic compounds are plant-derived secondary metabolites, biosynthesized mainly for protection against stress and oxidative damage and transferred via the nectar to the honey. The intensity of the color of a honey may be associated with the antioxidant strength of the honey. The phenolic, flavonoid, and carotenoid content is increased in darker honeys and reduced in lighter more transparent honey. As a result, biological properties, such as antioxidant activities and antibacterial capabilities, of the honey are related to its color, and darker honey tends to have enhanced properties [4, 15].

The most common phenolic compounds are phenolic acids and flavonoids [16]. Phenolic acids constitute an important class of phenolic compounds with bioactive functions typically found in vegetable products and foods. Also are secondary metabolites required for normal operation of naturally occurring plants. They can be divided into two subgroups according to their structure: the hydroxybenzoic and hydroxycinnamic acids. Acids derived from hydroxybenzoic acids include þ-hydroxybenzoic, vanillic, syringic, salicylic (2-hydroxybenzoate), gallic, and ellagic. These compounds might be existing in soluble form in cells, along with sugars or organic acids, or formed with cells linked to lignins. Hydroxycinnamic acids occur normally in their conjugated form as esters of hydroxy acids such as tartaric acid and shikimic as well as in their pure form, including þ-coumaric, caffeic, ferulic, and sinapic acids. Flavonoids (flavones, flavonols, flavanones, flavanols, anthocyanidin, isoflavones and chalcones) are the largest group of plant phenolic compounds. This group represents over 50% of all naturally occurring phenolic compounds. They are generally distributed in the seeds, bark, leaves, and flowers of plants and trees. In plants, these compounds give protection, against pathogens, herbivores, and UV radiation [5, 17].

The qualitative and quantitative difference in the phenolic profile of honey according to the different botanical sources represents the scientific basis of the two main lines of research about the study of honey phenolic fraction. The first approach is focused on the evaluation of the bioactive properties of honeys while the second approach attempts to attribute the botanical and/or the geographical origin of honey based on the existence and the abundance of at least one or more specific phenolic compounds, thus proposed as chemical marker(s) of origin. The results of these research studies are relevant in both directions; honey of varying botanical origins show a wide range of health-promoting properties like antibacterial, antiinflammatory, antioxidant, and radical-scavenging activity [4, 16, 18]. A wide range of phenolic constituents are present in honey such as quercetin, caffeic acid, caffeic acid phenethyl ester (CAPE), acacetin, kaempferol, galangin, chrysin, pinocembrin, pinobanksin, and apigenin, which have promising effects in the treatment of some diseases [19, 20].

Pinocembrin, pinobanksin, and chrysin are the characteristic flavonoids of propolis and these flavonoids have been found in European [19, 21] and Chilean honey samples [22]. Small amounts of propolis might be incorporated into honey; therefore, propolis flavonoids might contribute to the phenolic composition of honey. In temperate areas, the main sources of propolis are poplar (*Populus*) bud exudates. The identification of propolis-derived compounds like pinocembrin and chrysin could have an important contribution to the phenolic composition and antioxidant activity in corcolen (*Azara petiolaris*) Chilean honey [22].

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Antioxidant, antiinflammatory, antimicrobial, antiviral, and antiulcerous action, and the capability for regulating enzymatic browning are some of the principal characteristics of honey primarily attributed to phenolic compounds. The implementation of honey polyphenols has recently gained great interest from the functional food, nutraceutical and pharmaceutical industries. However, the efficacy of polyphenols relies on the preservation of their stability and bioactivity. Phenolic compounds, as well as other organic compounds, are degraded depending on the environmental conditions to which they are subjected. Spanish honey samples were subjected to liquefaction and liquefaction/pasteurization and the phenolic compounds evaluated as to the impact of industrial heat treatment. Phenolic compounds found in these honey samples were caffeic and þ-coumaric acids and flavonoids naringenin, hesperetin, pinocembrin, chrysin, galangin, quercetin, and kaempferol. A significant decrease in the concentration of galangin, kaempferol, myricetin, and þ-coumaric acid was observed after heat treatment [23]. Moreover, some flavonoid glycosides present in honey demonstrate certain instability under slight alkaline conditions and high sensitivity to oxidation in the presence of slight oxidizing agents such as hydrogen peroxide, which is present in honey and is responsible for the degradation verified in the flavonoids analyzed [24, 25].

The complexity of a food matrix like honey implies that the target analytes are usually present in low concentrations, and this demands the adoption of a multistep analytical procedure able to provide a careful measurement of these quantities [16]. Procedures using Amberlite XAD-2 columns for cleaning the complex matrices of honey and isolation of their phenols are often performed. In some cases, this step would reduce the need for sample manipulation and give a sample extract uniformly enriched in all components of interest and free from interfering matrix components. In these procedures, aqueous- acidified honey solutions are passed through the columns to retain phenols in sorbent beds and afterward eluded with methanol [19, 26]. These extracts are widely employed in analytical methods, biological assays, and functional food development, since the presence of sugars gives the entire honey a syrupy texture, which causes difficulties for some analysis and preparations. However, recoveries of phenolic acids and flavonoids extracted from deionized water (pH 2) using Amberlite XAD-2 demonstrated different recovery percentages, probably depending on the structure of the phenols studied. Kaempferol, *p*-coumaric acid, and syringic acid were completely adsorbed, but the recovery of gallic acid, caffeic acid, and quercetin by methanol is much less efficient [13, 27–29].

6. Abscisic acid

Abscisic acid, a plant hormone related to the protection of plants in environmental stress conditions, has been detected in corcolen and quillay honeys. The existence of abscisic acid in nectar is well established and is affected by environmental conditions, which might regulate the biosynthesis of certain secondary metabolites, such as phenolic compounds and abscisic acid. The biosynthesis of these compounds may be stimulated by plants, lowering damages through their capacity to capture free radicals under stress conditions, and reduce the penetration of UV-B ultraviolet radiation. The representation of these compounds in honey produced from *Q. saponaria* may be associated with the high interannual variability of climate conditions of the central zone of Chile [13, 30].

7. Biological activities

Clinical investigations of the therapeutic potential of honey are gradually growing and scientific evidence for the efficacy of honey in some conditions is beginning to emerge. The healing effect of honey could be classified by its antiinflammatory, antibacterial, and antioxidant properties of its components. Furthermore, honey has been reported to be effective in gastrointestinal disorders, in healing of wounds and burns, and in treating venous ulcers [31].

8. Antioxidant activity

Over many years, honey from different parts of the world have been shown to be one of the highest potential natural products in which phenolics, flavonoids, ascorbic acids, and some enzymes serve as potent antioxidants [32]. The antioxidant properties of honey are derived from both enzymatic (e.g., catalase, glucose oxidase, and peroxidase) and nonenzymatic substances (e.g., phenolic compounds, ascorbic acid, α-tocopherol, carotenoids, amino acids, proteins, and Maillard reaction products). The quantity and kind of these antioxidants are mainly dependent on the floral source. The main functional components of honey are flavonoids. They contribute significantly to the total antioxidant activity of honey and they act by several mechanisms including direct trapping of reactive oxygen species, inhibition of enzymes responsible for producing superoxide anions, chelation of transition metals involved in processes forming radicals, and prevention of the peroxidation process by reducing alkoxyl and peroxyl radicals [5, 15, 29]. The antioxidant activity of flavonoids in the majority of cases relies on the number and position of hydroxyl groups, additional substituents, and the glycosylation of flavonoid molecules. The presence of specific hydroxyl groups in the flavonoid rings improves antioxidant activity. Substitution patterns in the A ring and B ring, and the 2,3-double bond (unsaturated) and 4-oxo group in the C ring affect the antioxidant action of flavonoids as well. The glycosylation of flavonoids reduces their antioxidant activity compared to the analogous aglycones [5, 33].

These antioxidants may help to protect cellular damages from oxidative stress and lower the risk of chronic diseases. Furthermore, in recent years, there has been an increase in new methods for the research of free radicals and antioxidants in relation with advances in human health. Various studies have demonstrated that neuronal and behavioral changes occur with ageing, including in the absence of degenerative disease. Current studies indicate that dietary intake of antioxidant nutrients and cognition is closely related. Evidence from epidemiological, experimental and clinical studies demonstrates that the consumption of foods with high levels of dietary antioxidants might prevent or lower the risk of cognitive deterioration [34]. Many research models have been established in chemical and/or biological systems for the studies of mechanisms of action of antioxidants. Generally, antioxidant ability was measured and presented as total antioxidant capacity (TAC) [35, 36], total antioxidant potentials (TRAP) [37, 38], Trolox equivalent antioxidant capacity (TEAC) [39], ferric reducing/antioxidant power (FRAP) [40], and oxygen radical absorption capacity (ORAC) [41]. Mechanistically, these methods are based on either a single-electron transfer reaction or a hydrogen atom

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transfer reaction from an antioxidant or oxidant to a free radical. The total antioxidant activity is related to the radical scavenging ability and reductive activity [42].

Montenegro et al. [11] studied the antioxidant activity of unifloral honeys (quillay, ulmo, avellana, tiaca) of native plants from Chile. In this study, was observed an important correlation between total phenolic content and antioxidant activity evaluated by ferric reducing activity power—FRAP method. The ferric reducing activity power assay directly measures antioxidants with a reduction potential below the reduction potential of the Fe^{3+}/Fe^{2+} couple and the reaction is reproducible and linearly related to the molar concentration of the antioxidant(s) present in the sample. Furthermore, some variations in antioxidant activity between honey samples with the same botanical origin were observed. This variability could be explained by different accompanying species and geographical origin zone of the honey [11].

The scavenging activity towards peroxyl/alkoxyl radicals (ORAC method) is one of the most employed assays. In fact, in the last years databases of the ORAC index of foods have been built to emphasize the benefits of establishing the antioxidant capacity of polyphenol-rich foods [43]. The method is based on the ability of antioxidants to prevent the consumption of a target molecule mediated by free radicals generated during the aerobic thermal decomposition of AAPH (2,2′-azo-bis(2-amidinopropane). The target molecules are most commonly used are beta-phycoerythrin, fluorescein, and pyrogallol red. The use of the pyrogallol red (PGR) as probe is related to the amount and reactivity of a given phenolic compound towards the free radicals generated in the AAPH (2,2′-azo-bis(2-amidinopropane) dihydrochloride) thermolysis. The ORAC-PGR index can be considered as a measure of the capacity of the sample to remove peroxyl and alkoxyl radicals [44]. In complex mixtures, concentration, chemical nature, and possibly the interaction between the antioxidants present in the sample determine this index.

Recently obtained results from our research group demonstrated that quillay honey's ORAC-PGR index is not correlated to phenolic compound content present in samples but is highly correlated to flavonoid content. This is due to the fact that flavonoids are the polyphenolic species to which the highest reactivity is attributed [45]. In addition, the ORAC-PGR index in honey of *A. petiolaris*, named commonly as corcolen honey, collected from honeybee colonies of the central zone of Chile was evaluated. The value of this parameter for these samples is correlated to the percentage of corcolen pollen present, meaning that mostly the phenolic compounds and compounds with scavenger capacity belong to corcolen species. These correlations are quite interesting since just by means of the melissopalynological assay there could be evidence of the phenolics composition as well as the antioxidant capacity of monofloral *Azara sp*. honey [22].

Finally, more recently, our group has obtained results indicating that honey quillay compounds are reactive toward hypochlorite (HOCl). Hypochlorite has an important role in defense mechanisms that take part in the immune response toward microorganisms. However, it has also documented that hypochlorite, in certain pathophysiological conditions, can damage macromolecules including proteins, DNA, RNA, and cell membrane lipids, changing their biological function. The consumption of PGR-induced by hypochlorite is inhibited by compounds able to react with this reactive species. PGR-hypochlorite indexes obtained for quillay honey samples indicated high hypochlorite-mediated oxidation protection potential, these results being comparable to those obtained via Trolox—water-soluble vitamin E analogue (unpublished results).

9. Antibacterial activity

The broad-spectrum of antimicrobial activity of honey was demonstrated in various studies and reportedly exerts both bacteriostatic and bactericidal activities. The antimicrobial nature of honey depends on different factors acting singularly or synergistically, the most significant of which are phenolic compounds, pH of honey, H_2O_2, wound pH, and osmotic pressure exerted by the honey itself [15, 46]. The antibacterial capabilities of different unifloral Chilean honey, including ulmo honey (*E. cordifolia*), quillay honey (*Q. saponaria*), avellano honey (*G. avellana*), and tiaca honey (*C. paniculata*) were analyzed (**Table 2**). The methanolic extract of these honeys, obtained using Amberlite XAD-2 column, demonstrated better antibacterial capabilities than the honeys themselves, indicating an important role of the phenolic compounds in this activity. In *in vitro* assays, all of the honey extracts were able to inhibit the growth of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Streptococcus pyogenes* determining minimal bactericidal concentration [4, 11].

Floral Origin	Antioxidant/Antibacterial activities	Reference
Quillay honey (phenolic extracts)	Antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus typhi, S. aureus, Streptococcus pneumoniae, Vibri cholerae* and antifungal activity against *Candida albicans*.	Montenegro et al. [11]
Quillay honey (entire honey and phenolic extracts)	Oxygen radical absorbance capacity (ORAC-PGR) index related to the capacity of the sample to remove peroxyl and alkoxyl radicals	Bridi et al. [45]
Ulmo honey	Comparison of the antimicrobial activity of ulmo and manuka honey against methicillin-resistant *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*.	Sherlock, et al. [14]
Ulmo honey (phenolic extracts)	Inhibition of *in vitro* growth of human pathogenic bacteria *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and fungicidal activity on fungi genera *Mucor, Rhizopus, Aspergillus, Candida* and *Penicillium*	Montenegro and Ortega [48].
Ulmo honey	Ulmo honey topical application supplemented with ascorbic acid improves regeneration in burns in guinea pig.	Schencke, et al. [49–51]
Ulmo honey	Clinical trial: topical treatment using ulmo honey associated with oral ascorbic acid showed excellent clinical results for the healing of venous ulcers	Calderon et al. [52]
Ulmo, quillay avellana and tiaca honeys	Ferric reducing antioxidant power	Montenegro et al. [11]
Corcolen honey	Oxygen radical absorbance capacity (ORAC-PGR), index related to the capacity of the sample to remove peroxyl and alkoxyl radicals	Giordano et al. [22]

Table 2. Review of antioxidant and antibacterial activity in unifloral Chilean honey.

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The manuka honey derived from the manuka tree (*Leptospermum scoparium)*, which grows as a shrub, or a small tree throughout New Zealand and eastern Australia is the best known of the honeys. It has been reported to have an inhibitory effect on around sixty species of bacteria, including aerobes and anaerobes, positives, and Gram-negatives. The antimicrobial activity exhibited against pathogenic bacteria such as *S. aureus* make this honey a promising functional food for the treatment of wounds. The potential of honey to assist with wound healing has been demonstrated repeatedly and the healing properties can be ascribed to the fact that it offers antibacterial activity, maintains a moist wound environment that promotes healing, and has a high viscosity that helps to provide a protective barrier to prevent infection [47]. A study compared the antimicrobial activity of the ulmo honey with manuka honey against five strains of methicillin-resistant *S. aureus, E. coli*, and *P. aeruginosa*. The ulmo honey had greater antibacterial activity against all methicillin-resistant *S. aureus* isolates tested than manuka honey and similar activity against *E. coli* and *P. aeruginosa* using agar diffusion assay. The minimum inhibitory concentration assay showed that a lower minimum inhibitory concentration was observed with ulmo honey than with manuka honey for all five methicillin-resistant *S. aureus* isolates. For the *E. coli* and *Pseudomonas* strains, equivalent minimum inhibitory concentration was observed. Due to its high antimicrobial activity, ulmo honey may warrant further investigation as a possible alternative therapy for wound healing [14]. In Chile, ulmo honey extract has been patented for its bactericidal and fungicidal properties [48]. The document relates to uses of an extract of unifloral ulmo honey, rich in phenolic compounds, able to inhibit the *in vitro* growth of human pathogenic bacteria such as *S. aureus, P. aeruginosa, and E. coli*, in addition to exhibiting fungicidal and fungistatic activity on fungi genera *Mucor, Rhizopus, Aspergillus, Candida*, and *Penicillium*.

The use of ulmo honey in association with oral vitamin C as an alternative in healing treatment of *burn wounds* in guinea pigs (*Cavia porcellus*) improves regeneration in this type of wound and also reduces the possibility of infection, inflammation, and edema [49–51]. In addition, the clinical effect of topical treatment with ulmo honey associated with oral vitamin C in patients with venous ulcers was evaluated. This treatment method presented significant results, healing wounds faster in 100% of patients with all types of venous ulcers. Furthermore, the honey presented nonadherent and debriding properties was straightforward to apply and remove, and was well received by users [52].

Regarding honey of *Q. saponaria*, the antibacterial and antifungal activities were analyzed. Extracts of unifloral honeys of quillay were tested for antibacterial activity on *P. aeruginosa, E. coli, Staphylococcus typhi, S. aureus, Streptococcus pneumoniae* type β, and *Vibrio cholerae*, and antifungal activity against *Candida albicans*. The best *in vitro* activity of these extracts were on *S. aureus* and hemolytic *S. β*, both of which affect the skin [13]. The antibacterial effects exhibited could be related to an overall effect of the phenolic compounds present in the extract (caffeic, coumaric and salicylic acids, the flavanone naringenin and the flavonol kaempferol), which were detected by high-performance liquid chromatography.

Microbial resistance to honey has never been reported which makes it a very promising topical antimicrobial agent against the infection of antibiotic-resistant bacteria and in the treatment of chronic wound infections that do not respond to antibiotic therapy. The potency of honey, such as Chilean honey, against microorganisms suggests its potential to be used as an alternative therapeutic agent in certain medical conditions, particularly wound infection.

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