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Analysis of volatile compounds in three unifloral native Chilean honeys

Análisis de compuestos volátiles en tres mieles monoflorales nativas de Chile

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Abstract. Three unifloral honeys were identified by the pronounced presence of specific compounds by means of sensorial analysis and SPME-GC-MS. Smoky and resinous ('propolis') odors characterized unifloral "quillay" (*Quillaja saponaria*) honey. "Corontillo" (*Escallonia pulverulenta*) honey was characterized by caramel and vanillin aromas, and "ulmo" (*Eucryphia cordifolia*) honey by having an anise scent with a floral jasmine note. Safranal was a useful marker for "corontillo" honey. Isophorone and cetoisophorone were the distinctive compounds of unifloral "ulmo" honey. In "quillay" honeys, megastigmatrienone, 2-*p*-hydroxyphenylalcohol and minor quantities of β-pinene and linalool oxide were correlated with their sensory properties such as resinous.

Key words: : Ulmo honey, *Escallonia* or "corontillo" honey, *Quillaja* honey, SPME-GC-MS analysis.

Resumen. Tres mieles monoflorales se identificaron por la presencia notable de compuestos específicos usando análisis sensorial y SPME-GC-MS. Los olores a humo y a resina (o a propóleos) tipificaron la miel de quillay (*Quillaja saponaria*). La miel de corontillo (*Escallonia pulverulenta*) se caracterizó por sus aromas a caramelo y a vainilla, y la miel de ulmo (*Eucryphia cordifolia*) por su fragancia anisada con una nota floral de jazmin. Safranal constituyó un marcador útil para la miel de corontillo, mientras que isoforona y cetoisoforona fueron los compuestos distintivos de la miel monofloral de ulmo. En las mieles de quillay se correlacionaron megastigmatrienona, 2-p-hidroxifenilalcohol y las trazas de β-pineno y óxido de linalool con sus propiedades organolépticas tales como resinosa.

Palabras clave: miel de ulmo, miel de corontillo o de *Escallonia*, miel de *Quillaja*, análisis SPME-GC-MS.

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INTRODUCTION

Honey aroma has been studied for years. The composition of the honey's volatile fractions derives from its floral origin, and from the bee foraging habits and physiology. To date, six hundred compounds have been identified by gas chromatography mass spectrometry (GC/MS). As unifloral honeys differ with respect to their sensory properties, it is likely that their classification by chemical analysis is possible (Bogdanov et al., 2004). Radovic et al. (2001) identified several markers for the determination of honey's floral origin, and *Lavandula, Abies*, *Eucalyptus*, *Taraxacum* and *Brassica* honeys have been identified using GC/MS (Bouseta et al., 1992; Piasenzotto et al., 2003; Ruoff et al., 2005). Another technique, solid phase microextraction (SPME), has been used to identify *Robinia*, *Castanea*, *Tilia*, and *Thymus* honeys (Vittali & Guidotti, 1998; Tsigouri & Passaloglou-Katrali, 2004; Krist et al., 2004). Many volatile compounds are restricted to unifloral honeys and have been used as markers (Verzera et al., 2001; Perez et al., 2002; Piasenzotto et al., 2003; Ruoff & Bogdanov, 2004). Recently, Radovic et al. (2001) identified 110 compounds in 43 certified honeys, and Bentivenga et al. (2004) found the presence of hydrocarbon contaminants caused by the emissions from an oil refinery plant in Italian honeys. The aim of this work was to correlate the sensory properties of three types of unifloral Chilean honeys with authentic markers from GC/MS analysis. Study honeys were "quillay" (*Quillaja saponaria* Molina), "corontillo" [*Escallonia pulverulenta* (Ruiz et Pav.) Pers.] and "ulmo" (*Eucryphia cordifolia* Cav.). In Argentina, one of the major World honey producers, fetid honeys have been reported due to presence of *Discaria Americana* Hill. et Hook., *Scutia buxifolia* Reissek (Rhamnaceae) and *Acicarpha tribuloides* Juss. (Calyceraceae). These plant species are not in the Chilean flora, and other Rhamnaceae are not important in the pollen frequencies of unifloral honeys from Central Chile (Telleria et al., 2004). On the other hand, Argentine unifloral honeys differ from the Chilean ones, e. g. *Tessaria*, *Ziziphus* and *Eugenia*. Furthermore, *Schinus areira* L. (Peruvian pepper) has been reported as a honey-producing species from Argentina. However, this cultivated tree in Chile does not appear in the pollen frequency profiles both for uni- and multifloral honeys in that country (Cabrera, 2006; Colaneri et al., 2007). Also, there is a report of chollynergic intoxication due to ingestion of Venezuelan honeys, which include alkaloids from *Datura* o *Brugmansia*. This problem is not found in both the multi- or unifloral honeys from Chile (Vit & Barrera, 2002).

MATERIALS AND METHODS

Sensorial analysis. Thirteen honey samples were assessed. Samples were taken from either Matorral or Valdivian temperate forest ecoregions, and were collected in 2004 and 2005. From them, three endemic unifloral honeys were sampled for further analysis. Determination of their botanical origin followed Chilean Norm "NCh2981-2005" (Montenegro et al., 2008).

Sensorial analysis was carried out by a panel of 15 assessors or tasters, using the *scoring monadic* method in which each sample is assessed individually using a list of descriptors. Intensity of the perception of the descriptor was indicated from 0 to 9, where 0 corresponds to absence of perception of the descriptor. The descriptive terminology used followed Galan-Soldevilla et al. (2005) and Piana et al. (2004). New terms were introduced when necessary.

During the first nine weeks, the panel was trained by ranking honey samples using the different descriptors. An analysis of multiple comparisons of least significant difference (LSD) was used to determine whether the descriptors used varied significantly or not in the honey samples. Once all the descriptors showed significant differences within the universe of the samples studied, the panel was trained in five sessions, by scoring the intensities of the attributes of the different samples. The discriminative power of each attribute was assessed by ANOVA analysis. Once it was determined that all attributes were discriminant (values <0.05), the formal sessions began with the recognition of standards and honeys typified using melisopalynology. Results were RANOVA analysed using the software Statgraphics®, to determine the discriminative capacity of the descriptors, repeatability, and the consistency of the panel. A principal components analysis (PCA) (Senstools v.3.0®) was subsequently carried out with the descriptors to produce a sensorial chart, and a profile for each of the samples.

Chemical analysis. Three certified unifloral Chilean honeys were selected for further chemical analysis and kept in a cold chamber: "ulmo" (*E. Cordifolia*; sample numbers 335: from 10th Region); "quillay" (*Q. Saponaria*; 337: from Santiago Metropolitan Region) and "corontillo" (*E. Pulverulenta*; 329: from 4th Region).

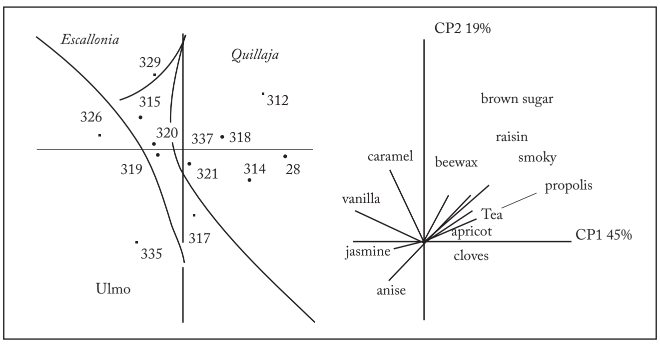
For each sample, 10 g of honey were placed in a 10 ml vial, to which 0.5 g anhydrous sodium sulfate and 25 µl of internal standard (4-nonalol solution 3.568 mg/ml) were added. The vial was shaken for 30 minutes at 70°C for pre-conditioning. A carboxen/polydimethylsiloxane (CAR/PDMS) SPME fiber 75 µm in diameter, contained within a support, was also pre-conditioned at the same temperature for 20 minutes, and then quickly transferred to the injector of the GC/MS (Gas chromatograph Hewlett-Packard model 6890, coupled with a mass spectrometer Hewlett-Packard model 5972®). For the chromatographic analysis, the injector and detector temperatures were 250°C. The column [DB – WAXETR Fused capillary column; polyethylene glycol, 60 m; 250 µm i.d.; 0.25 µm film thickness (J&W Scientific® 122-7362)] was maintained at 40°C for 5 minutes. It was thereafter heated at a rate of 3°C per minute until reaching 240°C, and then maintained at this temperature for 10 minutes. The gas flow rate was 20 ml/min and the carrier gas was helium. Compounds were quantified using automatic peak area calculation, and partially identified using correlations between retention times and resident library.

RESULTS

Sensorial analysis. Few descriptors were found to be discriminative; nevertheless the recognition capacity and consistency of the panel were reproducible. Aromas smelt were: smoky, raisin and resinous propolis in "quillay" honey, caramel and vanilla in "corontillo" honey, and anise and jasmine in "ulmo" honey.

The PCA (Fig. 1) visualizes the relationship between the attributes and samples. The principal component 1 (PC1) explains a 45% of the original variability in the data, and the PC2 explains 19%. It can be appreciated that samples towards the upper right section of the graph are mostly characterized by the attributes smoky and resinous (propolis), while those towards the upper left section are characterized by the attributes vanilla and caramel.

Fig. 1. Principal components analysis (PCA) of Chilean uni- and multifloral honeys.
Fig. 1. Análisis de componentes principales (PCA) de mieles chilenas mono- y multiflorales.



Chemical analysis. Major compounds of the three unifloral honey samples were phenolic derivatives, terpenes, and norisoprenoids (Tables 1, 2 and 3). From the aromatic point of view, it represents the most interesting group of honeys. "Ulmo" honey contained a large amount of terpenes in the form of lilac aldehyde and lilac alcohol. The major compounds in "corontillo" and "quillay" honeys were phenolic compounds. The presence of safranal was exceptional in "corontillo" honey and could be established as a diagnostic character. Isophorone and cetoisophorone were correlated by the assessor descriptors as spice aroma in the unifloral "ulmo" honey (Ampuero et al., 2004). Acetophenone contents, which were sensorially diagnosed as a *Gevuina avellana* Molina scent, corresponded to amounts found in honeys of other botanical palynological origins (Ampuero et al., 2004); it means that this marker is of no diagnostic value.

Table 1. SPME-GC/MS composition of three unifloral Chilean honeys.
Tabla 1. Composición de tres mieles Chilenas monoflorales por SPME-GC/MS.

Composition	Honey Sample number		
Norisoprenoids (mg/kg)	335*	329	337
isophorone	6.99	0.30	nd
cetoisophorone	5.10	1.07	nd
safranal	nd	1.05	nd
trans β-damascenone	1.09	3.72	0.19
3-hydroxy-5, 6-epoxy-β-ionone	0.27	0.50	nd
3, 4-dehydro-β-ionone	0.14	nd	nd
3, 5-dehydro-β-ionone	nd	0.33	nd
megastigmatrienone	nd	nd	0.53
megastigma-5,7,9-trienone	nd	nd	0.24
TOTAL	**13.95**	**6.97**	**0.96**

*Honey sample number: "ulmo" honey (335), "corontillo" honey (329), and "quillay" honey (337)
*Número de muestra de miel: miel "ulmo" (335), miel "corontillo" (329), y miel "quillay" (337).

Table 2. SPME-GC/MS composition of three unifloral Chilean honeys.
Tabla 2. Composición de tres mieles Chilenas monoflorales por SPME-GC/MS.

Composition	Honey Sample Number		
Terpenes (mg/kg)	335	329	337
α felandrene	0.02	nd	nd
iso terpinolene	0.03	0.84	nd
α pinene	nd	nd	0.19
β pinene	nd	nf	0.38
limonene	0.12	0.32	nd
sabinene	0.05	nd	nd
camphene	0.10	nd	nd
linalool oxide	0.21	0.36	0.25
linalool	nd	nd	0.15
hotrienol	0.56	0.85	0.09
myrtenol	0.26	0.09	nd
neryl acetone	0.14	0.37	nd
eucarvone	0.46	nd	nd
lilac alcohol	34.54	nd	nd
lilac aldehyde	36.13	nd	nd
trans anethol	1.11	nd	nd
8-hydroxy-6,7-dihydrolinalool	2.03	nd	nd
p-cymene	0.04	0.10	nd
cis rose oxide	nd	0.19	0.11
nerol	nd	0.06	nd
α calacorene	nd	0.07	nd
cumene	nd	0.05	0.20
cadalene	nd	0.10	nd
TOTAL	**75.79**	**3.39**	**1.37**

Table 3. SPME-GC/MS composition of three unifloral Chilean honeys.
Tabla 3. Composición de tres mieles Chilenas monoflorales por SPME-GC/MS.

Composition	Honey Sample Number		
Phenolic compounds (mg/kg)	335	329	337
1-methoxy-4-methyl-bencene	1.53	0.43	0.04
phenol	0.24	0.16	0.35
4-vinyl-guaicol	0.08	0.78	0.04
diphenyl-acethaldehyde	0.37	nd	nd
methyl-4-methoxybenzoate	0.18	0.22	nd
m-acethyl-acethofenone	0.11	nd	nd
4-vinyl-phenol	0.14	0.17	0.13
2, 4, 6-trimethyl-acetofenone	2.24	nd	nd
eugenol	nd	0.53	nd
iso-eugenol	nd	0.11	nd
trans-β-ocymene	nd	0.16	nd
methyl salycylate	nd	0.23	nd
p-sec-buthylphenol	nd	0.21	nd
3-picoline	nd	1.0	nd
syringol	nd	0.24	nd
2, 6-dimethoxy-phenol	nd	nd	0.28
2-*p*-hydroxyphenylacohol	nd	nd	0.41
guaicol	nd	nd	0.03
2(*p*-methoxyphenyl)-ethanol	nd	nd	0.26
vainillin	nd	0.77	nd
TOTAL	**4.89**	**5.01**	**1.54**

DISCUSSION AND CONCLUSIONS

Establishment of a sensorial panel is an advance in the implementation of long term strategies which provide objective tools to characterize honey aromas of known botanical origin. In this research, an analytical system of chemical assessment was used complementarily to identify three unifloral Chilean honeys. This followed the idea of Pianna et al. (2004), who suggested the need to improve sensorial analysis using more analytical methods.

Unifloral "ulmo" honey was easily recognized, and was typified as having a jasmine scent. In sensorial terms it was characterized as floral, but also as having an anise or spice aroma. Isophorone and cetoisophorone were correlated by the assessor descriptors as spice aroma (Rowland et al., 1995; Ampuero et al., 2004). Phenylacetaldehyde, nonanoic acid, acetophenone, decanoic acid, benzaldehyde, phenylacetonitrile, isophorone, and nonanal have been detected in thyme (*Thymus vulgaris* L.) (Alissandrakis et al., 2007). The *cis*-linalool was detected in *Acacia*, 1-octene or 2, 3-pentanedione nonanol, nonanal, and nonanoic acid in *Eucalyptus* (Cuevas-Glory et al., 2007), dimethyl sulphide in *Brassica*, and 1-*p*-menten-9-al and lilac aldehyde in *Citrus* (Cuevas-Glory et al., 2007). A saffron (*Crocus sativus* L.) aroma, correlated with the presence of safranal, was characteristic of the "corontillo" honey. It is the first time that a safranal scent has been reported in honey. In contrast, "quillay" honey was not well defined. The assessors have difficulties in recognizing it, and it occupied an ample sector in the PCA (samples 337, 314 and 317). This may indicate that the samples are from three different sorts of monofloral "quillay" honeys. However, it may also be due to the fact that the honeys contain other floral influences. Persano Oddo & Bogdanov (2004) have recently claimed "no honey is exactly the same as another". Rather, there is a gradient in the number and percentages of different pollen types from unifloral to multifloral honeys. Various definitions have been described for unifloral honey by different authors (Ruoff & Bogdanov, 2004). However, a universal definition does not yet exist. A better characterization of "quillay" honey might be achieved by further comparative analysis with essential oils from native floral resources. This might produce aromas closer to the natural identities of the Chilean productive zone.

Multifloral honeys have diverse profiles without particular characteristics. This indicates that only unifloral honeys with defined aromatic characteristics present an opportunity to generate unique products. Unifloral "corontillo" honey, for example, has great potential. This is because of its fruity aromatic properties, which were due to its hotrienol content. Considering the ubiquity of our honeys, one to six markers are needed to identify them.

Research conducted by Rothe & Thomas (1963) on the aroma thresholds revealed that not all volatile compounds contributed to aroma. This implies a strategy change when searching for odour activity values or compounds of major sensorial activities. Using prepared synthetic samples, Grosch (2001) determined that no more than 5% of the sample's volatile compounds contributed to its aroma. For example, when *cis* oxide of rose was absent in the *Gewürztraminer* wine models, the aroma changed drastically and did not resemble that of the original samples. In contrast, the absence of β-damascenone and geraniol have less effect (Zhou et al., 2002). Wüst & Mosandl (1999) revised lime ether 2, 4, 5, 7a-tetrahydro-3, 6-dimethylbenz[b]furan isolated from both lime flowers and honey. It was found that these compounds were important chiral monoterpenoid ethers which can be enantioselectively analysed, and serve as controls for aroma authenticity.

Unifloral Chilean honeys were typified by chemical markers, being easy to characterize "ulmo" honey by its content of lilac aldehyde and alcohol. Phenolic content allowed identification of "quillay" and "corontillo" honeys, with a greater diversity in the first case, and a distinctive content of safranal in "corontillo" honey.

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