ΦYTON
REVISTA INTERNACIONAL DE BOTÁNICA EXPERIMENTAL
INTERNATIONAL JOURNAL OF EXPERIMENTAL BOTANY
FUNDACION ROMULO RAGGIO
Gaspar Campos 861, 1638 Vicente López (BA), Argentina
www.revistaphyton.fund-romuloraggio.org.ar

Volatile compounds of unifloral honey and floral nectar from *Quillaja saponaria*

Compuestos volátiles de miel monofloral y néctar floral de *Quillaja saponaria*

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Abstract. Currently, the search for chemical markers related to the botanical origin of honey is an important issue because of its potential use as a complementary tool for melisopalinological analysis. The objective of this research was to compare the (1) volatile compounds of *Quillaja saponaria* Mol. (Fam. Quillajaceae) floral nectar with those of unifloral honey of this same species, and (2) volatile compounds in *Q. saponaria* honeys from the same geographical origin. For the identification and semiquantification of volatile compounds, Gas Chromatography with Mass Spectrometry (GC-MS) was performed. The nectar of *Q. saponaria* presented volatile compounds different from the compounds identified in its unifloral honey, which may be precursors of the compounds present in *Q. saponaria* honey. Ten volatile compounds were found in all the samples of *Q. saponaria* honey: 2-methyl butyric acid (2 – 21.6 μg/L), benzyl alcohol (1 – 6 μg/L), 2-phenylethanol (16 – 125.3 μg/L), ketoisophorone (2.6 – 15.9 μg/L), linalool (2.4 – 13.8 μg/L) and its oxides 1 and 2 (6 – 13.3 μg/L and 3 – 7 μg/L, respectively), β-damascenone (4 - 12 μg/L), pantolactone (2 – 7.5 μg/L) and furfural (7 – 44,2 μg/L). These compounds were common in unifloral honey with different floral sources from other countries. These results would indicate that *Q. saponaria*honey does not present specific volatile compounds that allow its clear differentiation from other unifloral honey.

Keywords: Unifloral honey; Volatile compounds; SPME-GC-MS; *Quillaja saponaria*; Chile.

Resumen. Actualmente, la búsqueda de marcadores químicos relacionados con el origen botánico de las mieles es de gran interés debido a su uso potencial como una herramienta complementaria al análisis melisopalinológico. El objetivo de este estudio fue comparar (1) los contenidos de compuestos volátiles del néctar de *Quillaja saponaria* Mol. (Fam. Quillajaceae) con mieles monoflorales de esta misma especie, y (2) los compuestos volátiles de mieles de *Q. saponaria* recolectadas provenientes del mismo origen geográfico. Para la identificación y semicuantificación de compuestos volátiles se utilizó Cromatografía de Gases con Espectrometría de Masa (GC-MS). El néctar de *Q. saponaria* presentó compuestos volátiles diferentes a los identificados en las mieles, los que podrían ser precursores de los compuestos presentes en dichas mieles. Diez compuestos volátiles fueron encontrados en las cinco mieles de *Q. saponaria* analizadas: ácido 2-metil butírico (2 – 21,6 μg/L), benzil alcohol (1 – 6 μg/L), 2-feniletanol (16 – 125,3 μg/L), ketoisoforona (2,6 – 15,9 μg/L), linalool (2,4 – 13,8 μg/L) y sus óxidos 1 y 2 (6 – 13,3 μg/L y 3 – 7 μg/L respectivamente), β- damascenona (4 -12 μg/L), pantolactona (2 – 7,5 μg/L) y furfural (7 – 44,2 μg/L), siendo estos compuestos comunes en mieles monoflorales de distintas fuentes florales descritas en mieles de otros países. Estos resultados indicarían que las mieles de *Q. saponaria* no presentan un tipo particular de compuestos volátiles que permitan diferenciarlas claramente respecto de otras mieles monoflorales.

Palabras clave: Miel monofloral; Compuestos volátiles; SPME-GC-MS; *Quillaja saponaria*; Chile.

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Recibido / Received 20.XI.2012. Aceptado / Accepted 15.VII.2013.

INTRODUCTION

Honey aroma has been studied for several years due to its relation to organoleptic quality and authenticity (Pérez et al., 2002; Montenegro et al., 2008). Each unifloral honey has distinct aroma due to specific volatile compounds that may be derived from floral nectar; therefore, its organoleptic quality depends mainly on the origin floral source (Kaskoniene & Venskutonis, 2010). The differences in sensory properties of unifloral honey make possible to establish a relation between the main species present in honeys, and one or more compounds responsible for honey aroma and other volatile compounds identified by chemical analysis (Piasenzotto et al., 2003; Bogdanov et al., 2004; Manyi-Loh et al., 2011).

The volatile compounds could be used to determine the main floral source in unifloral honey, providing a tool to detect fraud by addition of pollen and misdescription of pollen grains (Radovic et al., 2001; Bogdanov & Martin, 2002; Arvanitoyannis et al., 2005). Furthermore, it has been reported that pollen grains of kanuka (*Kunzea ericoides*) and manuka (*Leptospermum scoparium*) are indistinguishable by melisopalinological analysis (Stephens et al., 2010); therefore, alternative tools have to be developed to identify the real botanical origin of this kind of unifloral honeys to guarantee honey authenticity.

This issue has been addressed in various studies, and several aromatic markers have been identified in different types of honey all over the world, like citrus honey from Greece, Spain and Italy; orange, strawberry-tree, eucalyptus and dandelion honey from Italy; salvia, false acacia and chestnut honey from Croatia; cambará honey from Brazil; fir, acacia and lavender honey from France and cotton honey from Greece. These analyses are commonly performed by Gas Chromatography/Mass Spectrophotometry (GC-MS) (Alissandrakis et al., 2003, 2005a, 2005b, 2007a, 2007b; Piasenzotto et al., 2003; Bentivenga et al., 2004; Bianchi et al., 2005; Moreira & De Maria, 2005; Jerkovic et al., 2006, 2007, Castro-Vázquez et al., 2007; Daher & Gülaçar, 2008).

In previous research, volatile composition analysis and sensory evaluation were performed on three types of native unifloral honey from Chile. This allowed the researchers to relate distinctive aroma descriptors in unifloral honey to the presence of terpenes, norisoprenoids, and phenolic derivatives. Nevertheless, researchers had difficulty recognizing aroma in *Quillaja saponaria* Mol. (Fam. Quillajaceae) honey, possibly due to the use of different types of unifloral *Q. saponaria* honey. However, it may also be due to the fact that the honey contains other floral influences (Montenegro et al., 2009).

Plant species have a particular chemical composition. Because honey comes from the flower nectar of certain species, finding a correlation between the chemical composition and the species that originated each particular honey would be expected.

Chile has a great diversity of native flora that can be used by bees (*Apis mellifera* L.) for honey production (Montenegro, 2002; Montenegro et al., 2010). Nevertheless, little information exists about the volatile compounds present in Chilean native honey, and information about the chemical composition of native floral nectar has not yet been described.

The objective of this research was to compare the (1) volatile compounds of *Quillaja saponaria* Mol. (Fam. Quillajaceae) floral nectar with those of unifloral honey of this same species, and (2) the volatile compounds of *Q. saponaria* honeys from the same geographical origin to establish possible volatile compounds as chemical markers for *Q. saponaria* honey.

MATERIALS AND METHODS

Nectar and honey samples. Floral nectar and honey samples were collected from four sampling sites (Petorca, Limache, Huayacán, and Alicahue) in the Valparaiso Region (32° 02' - 33° 57', V Region) in Central Chile (Fig. 1). Native vegetation was predominant in all of the sampling sites.

During *Q. saponaria* flowering (November and December), a representative number of trees were identified and labeled. Plants with leaf damage were discarded. Floral nectar from *Q. saponaria* flowers (250 flowers) was taken randomly from 10 trees. The floral nectar was collected with glass capillary tubes as described by Southwick (1983) and Vidal et al. (2006).

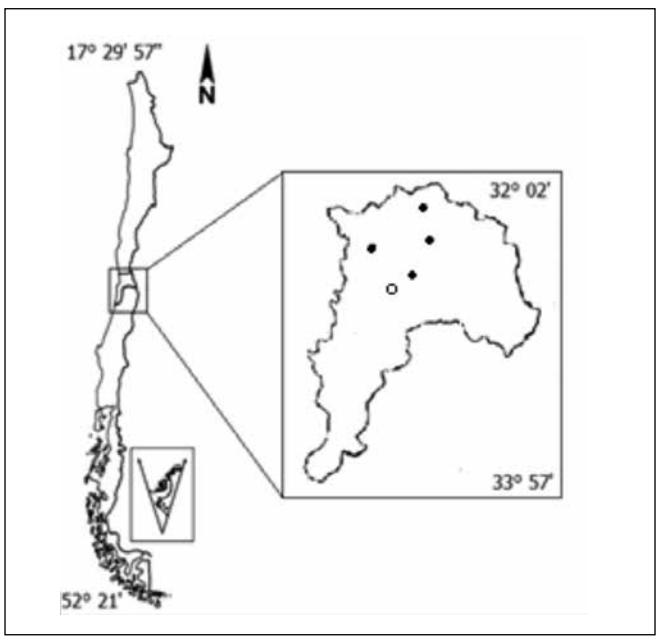

Fig. 1. Map of Valparaiso Region, Chile. Black circles indicate the origin of the *Quillaja saponaria* honey samples, the white circle indicates the origin of *Quillaja saponaria* nectar.

Fig. 1. Mapa de la Región de Valparaíso, Chile. Los puntos negros indican las zonas de origen de las muestras de miel de *Quillaja saponaria*, el círculo indica el origen del néctar de *Quillaja saponaria*.

During the corresponding harvest season, fifty-seven honey samples from the same geographical area were collected and studied using melisopalinological analysis to certify the *Q. saponaria* botanical origin as described in norm NCH 2981.OF2005 (Montenegro et al., 2008). The pollen grains were compared with a Pollen Grain Library for their identification. The data analysis was conducted using Statistical Analysis of Proportions, using maximum likelihood estimation to construct confidence intervals (Mead et al., 1993). Species that presented proportions lower than 0.03 (considering confidence interval) were discarded (Loveaux et al., 1978; Moar, 1985). Results and corresponding statistics are shown in Appendix 1 (Tables 6 to 62) (www.revistaphyton.fundromuloraggio.org.ar/vol83.html).

According to this analysis, five unifloral *Q. saponaria* honey samples were identified and selected for the chemical research. The botanical origin (percentage of *Q. saponaria*), and companion species of these honey samples are described in Table 1.

Table 1. Percentage of *Quillaja saponaria* presence in honey, and companion species.
Tabla 1. Porcentaje de presencia de *Quillaja saponaria* en miel, y especies acompañantes.

Honey	% *Quillaja saponaria*	Companion species*
1	81.5	*Brassica campestris* (8%)
2	70.5	*Shinus molle* (3%), *Gentianella ottonis* (12%)
3	55.3	*Trevoa quinquenervia* (24%), *Medicago polymorpha* (3.5%)
4	63.2	*Retanilla trinervia* (3.3%), *Brassica campestris* (4.2%), *Medicago polymorpha* (4.4%), *Trifolium repens* (5.4%), *Lotus corniculatus* (7.4%)
5	87.2	*Trevoa quinquenervia* (4.4%)

*At least 3% presence of companion species was considered.

Chemical analysis. One sample of floral nectar and five certified *Q. saponaria* honey samples were kept in a cold chamber before the chemical analysis. A 2 cm 50/30 µm DVB/Carboxen/PDMS StableFlex fiber (Supelco, Inc., Bellefonte, PA) was used for aroma extraction.

The honey solution plus sodium chloride (0.2 g) and internal standard (1 µL of 4-nonalol solution 3.460 mg/mL) were placed in a 20 mL vial tightly capped with a Teflon/silicone septum (catalog number S126-0020, I-CHEM). The sample was equilibrated at 35 °C in a water bath for 5 min and extracted after stirring for 1 hour at the same temperature. After extraction, SPME fiber was inserted into the injection port of the GC/MS to desorb the analytes. GC/MS analyses were carried out on a HP 6890 Gas Chromatograph, coupled to a 5972A MSD Hewlett Packard mass spectrometer, and equipped with a 60 m x 0.25 mm x 0.25 µm DB-WAXETR capillary column (J&W Scientific). The injector was set at 260 °C and column oven temperature was held for 5 min at 40 °C, then raised 3 °C/min until it reached 240 °C, at which point it was held for 25 min. Mass spectra was obtained by electron impact ionization (70 eV) scanning a mass range of 35-350 m/z. The MS quadrupole and MS source temperatures were 150 °C and 220 °C, respectively. The spectrometric data was compared with those from NIST-EPA-NIH libraries (http://www.nist.gov/srd/nist1a.htm) that have more than 130000 entries. Semiquantitative analysis was carried for the volatile compounds identified.

The repeatability of the extraction procedure was evaluated using nonalol as internal standard. The relative standard deviations (RSD) of the 10 common honey sample compounds are shown in Table 2.

Table 2. Relative standard deviation (% RSD) of 10 compounds obtained from a honey sample, estimated from 3 extractions.
Tabla 2. Desviación estándar relativa (% DER) de diez compuestos obtenidos de una muestra de miel, calculados a partir de 3 extracciones.

Compound	% RSD
Linalool oxide 1 (Z ó E)	21.6
Furfural	16.4
Linalool oxide 2 (Z ó E)	1.4
Linalool	11.8
2- Methyl-butyric acid	32.7
Ketoisophorona	21.1
β-Damascenone	3.8
Benzyl alcohol	11.4
2- Phenyl etanol	0.2
Pantolactone	28.1

RESULTS AND DISCUSSION

The main factors that affect the composition of volatile compounds in honeys are: floral source, geographical origin, honey maturity, and harvest season (Gheldof & Engeseth, 2002; Kaskoniene & Venskutonis, 2010). Botanical origin seems to be the main factor (Kaskoniene & Venskutonis, 2010). The five *Q. saponaria* honey samples were selected from the same geographical area (V Region of Central Chile) during the same season (2011-2012). For these reasons, differences in the content of volatile compounds in *Q. saponaria* honey might be attributed mainly to the honey samples companion species and postharvest conditions.

Different methods of extraction for the determination of volatile compounds in honey by GC-MS have been described (Alissandrakis et al., 2003; Bianchi et al., 2005; Castro-Vázques et al., 2010). The use of CAR/PDMS columns has been described as an effective fiber for the isolation of aromatic compounds in honey (Plutowska et al., 2011), and was selected for this research. The RSD of the 10 common compounds in the five honey samples were found in a range of 0.2 - 32.7%. Similar values have been described by Alissandrakis et al. (2003) and Piasenzotto et al. (2003). Nevertheless, our results showed higher RSD for 2- methyl-butyric acid and pantolactone.

Volatile compounds in *Q. saponaria* nectar and comparison to its unifloral honey. An example of a Chromatogram obtained by GC-MS of floral nectar and the corresponding volatile compounds is shown in Figure 2.

Floral nectar was mainly composed of esters in total concentrations of 189.0 µg/L; ethyl acetate, trimethyl acetate, buthyl acetate, methyl laurate, methyl caproate, ethyl hexanoate, methyl octanoate and methyl benzoate were identified. The group of second importance was terpenes, in concentrations of 80.5 µg/L; β-pinene, limonene, 1,8-cineole, cymenene 1 and 2 and thujone were identified (Table 4, Fig. 2). These results are the first report of *Q. saponaria* nectar volatile composition.

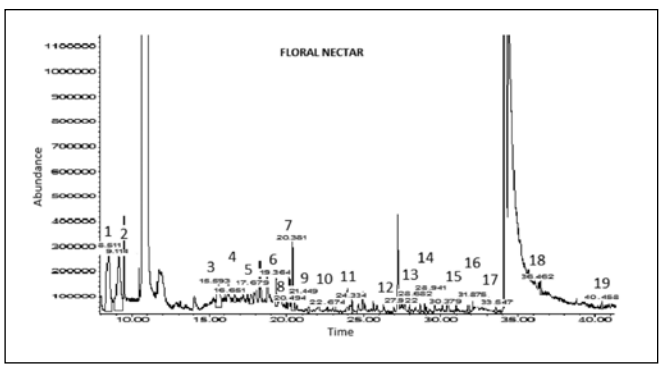

Fig. 2. GC-MS chromatogram of volatile compounds of *Quillaja saponaria* floral nectar. Peaks and corresponding retention time (RT): 1=ethyl acetate (RT: 8.511), 2=trimethyl acetate (RT: 9.114), 3= buthyl acetate (RT: 15.593), 4=β-pinene (RT: 16.651), 5=methyl laurate (RT: 17.679), 6=hexanal dimethyl acetal (RT: 19.364), 7=limonene (RT: 20.381), 8=methyl caproate (RT: 20.494), 9= 1,8-cineole (RT: 21.449), 10=ethyl hexanoate (RT: 22.674), 11=cymene 1 (RT: 24.334), 12=6-methyl-5-hepten-2-one (RT: 27.922), 13=cymene 2 (RT:28.682), 14=octanal dimethyl acetal (RT: 28.941), 15=nonanal (RT: 30.379), 16=thujone (RT: 31.875), 17=nonanal dimethyl acetal (RT: 33.547), 18=benzaldehyde (RT: 36.462), 19=methyl benzoate (RT: 40.458).

Fig. 2. Cromatograma de GC-MS de los compuestos del nectar de flores. Peaks y tiempo de retención (TR): 1= etil acetato (TR: 8,511), 2=trimetil acetato (TR: 9,114), 3=butil acetato (TR: 15,593), 4=β-pineno (TR: 16,651), 5=metil laurato (TR: 17,679), 6=hexanal dimetil acetal (TR: 19,364), 7=limoneno (TR: 20,381), 8=metil caproato (TR: 20,494), 9= 1,8-cineole (TR: 21,449), 10=etil hexanoato (TR: 22,674), 11=cymene 1 (TR: 24,334), 12=6-metil-5-hepten-2-one (TR: 27,922), 13=cymene 2 (TR: 28,682), 14=octanal dimetil acetal (TR: 28,941), 15=nonanal (TR: 30,379), 16=thujone (TR: 31,875), 17=nonanal dimetil acetal (TR: 33,547), 18=benzaldehído (TR: 36,462), 19=metil benzoate (TR: 40,458).

The volatile compounds identified in the *Q. saponaria* nectar differ both in the type and relative concentration when compared to the five unifloral honey samples (Table 3, 4 and 5). Nevertheless, there were two compounds (nonanal and benzaldehyde) found in both nectar and honey of *Q. saponaria.* Nonanal was found in higher mean concentrations in honey (9.8 µg/L) than nectar (3.8 µg/L), whereas bezaldehyde was found in higher concentration in nectar (9.6 µg/L) than honey (1.3 µg/L) (Table 5). These differences may be explained by the presence of compounds in nectar that could be precursors of other compounds present in honey, and agree with results obtained by other researchers (Rowland et al., 1995; Alissandrakis et al., 2003, Alissandrakis et al., 2007a, Alissandrakis et al., 2007b) where floral nectar and honey do not always share identical volatile compounds.

Comparison of volatile compounds in *Q. saponaria* honey. An example of Chromatograms obtained by GC-MS of each *Q. saponaria* honey, and the corresponding volatile compounds, are shown in Figure 3.

The volatile compounds identified in the five *Q. saponaria* honey samples differ both in type and relative concentration. In the five honey samples, 2-methyl butyric acid was found as the only type of acid in concentrations between 2.0 - 21.6 µg/L (Table 3). Among alcohols, benzyl alcohol and 2-phenylethanol were identified in concentrations between 1.0 - 6.0 µg/L and 16.0 - 125.5 µg/L, respectively (Table 3). Ketoisophorone was the only ketone identified in the five honey samples in concentrations between 2.6 – 15.9 µg/L (Table 4). Furans were identified in the form of furfural with concentrations that varied between 7.0 – 44.5 µg/L (Table 5). Linalool and oxide 1 and 2 were the only terpenes found in all five samples with concentrations that oscillated between 6.0 – 13.3 µg/L, 3.0 – 7.0 µg/L and 3.0 – 13.8 µg/L, respectively (Table 4). One norisoprenoid was identified in the form of β–damascenone in concentrations between 3.4 – 12.0 µg/L and one lactone was identified in the form of pantolactone in concentrations of 2.0 – 7.7 µg/L (Table 5).

These results could be explained by the different companion species in each honey. To be classified as a unifloral honey by melissopalinoligical analysis, at least 45% of the honey sample's pollen grain must come from a single species (Montenegro et al., 2008). The botanical origin results in this research indicate that the five unifloral honey samples had a *Q. saponaria* percentage of between 55.3 – 87.2%, and the companion species oscillated between 22.8 - 44.7% (Table 1). An important variety of the companion species was identified in the five honey

Table 3. Acid and alcohol composition (µg/L) of flower nectar and five Chilean unifloral honey samples of the same botanical origin (*Quillaja saponaria*) obtained via SPME-GC/MS.
Tabla 3. Composición de ácidos y alcoholes obtenidos mediante SPME-GC/MS de néctar de flores y cinco mieles monoflorales chilenas del mismo origen botánico (*Quillaja saponaria*).

	Nectar (µg/L)	Honey 1 (µg/L)	Honey 2 (µg/L)	Honey 3 (µg/L)	Honey 4 (µg/L)	Honey 5 (µg/L)
Acids						
Acetic acid	ND	ND	23	ND	5.5	32.1
Butiric acid	ND	ND	3	ND	ND	19.7
2-Methyl butiric acid	ND	2	15	6	2.9	21.6
Hexanoic acid	ND	ND	8	2	2.2	7.4
Propanoic acid	ND	ND	ND	ND	ND	3.5
Isobutiric acid	ND	ND	ND	ND	ND	4.7
Butanoic acid	ND	ND	ND	ND	1.9	15
Isovaleric acid	ND	ND	ND	ND	ND	18.9
Total		2	48	8	12.5	122.9
Alcohols						
3-Methyl-3-buten-1-ol	ND	ND	6	1	ND	ND
2-Heptanol	ND	1	3	2	ND	ND
Benzyl alcohol	ND	1	6	5	2.5	1.8
2-Phenylethanol	ND	23	35	16	55.7	125.3
4- Methoxyphenethyl alcohol	ND	13	ND	7	ND	ND
2-Methyl-3-buten-2-ol	ND	ND	ND	ND	ND	2.3
1-Hepten-4-ol	ND	ND	ND	ND	19	ND
1-Butanol	ND	ND	ND	ND	ND	0.8
4-Octanol	ND	ND	ND	ND	0.2	0.4
2-Ethyl-2-heptanol	ND	ND	ND	ND	1.8	ND
p-Methoxyphenethyl alcohol	ND	ND	ND	ND	13	33.9
Total		38	51	31	92.2	166.1

ND: Not detected

samples; native species (*Schinus molle, Gentianella ottonis, Trevoa quinquinervia, Retanilla trinervia*) predominated. According to our knowledge, the volatile compounds of these Chilean native species have not been described yet.

The nature of the honey used in most of this type of research makes it possible to easily correlate results of chemical composition among the different honey samples. This is because mostly cultivated species are used to obtain honey (citrus, lavender, eucalyptus, salvia, among others), which results in a very high percentage of the predominant species in each honey and a lower variability between honeys of the same botanical origin. This research, on the other hand, was focused on sampling sites where non-cultivated species (mostly native plants) predominated. This situation meant that there were a wider variety of species where honeybees collected nectar, resulting in a lower percentage of the predominant species.

Honey samples N° 1, 2, 4 and 5 had similar concentrations of β-damascenone (Table 5). This compound has been previously described in rhododendron, buckwheat, thyme and citrus honey (Castro-Vásquez et al., 2007; Alissandrakis et al., 2007 b; Kaskoniene & Venskutonis, 2010).

Honey samples N° 1, 2, 3 and 4 had similar concentrations of pantolactone (Table 5), linalool and its oxides (Table 4) and benzil alcohol (Table 3). Linalool and its oxides have been described in citrus, thyme, quillay, Christ thorn, cotton, chestnut, heather, rape, sunflower, lavender, false acacia and acacia honey (Radovic et al., 2001; Alissandrakis et al., 2005b; Castro-Vásquez et al., 2007; Jerkovic et al., 2007; Montenegro et al., 2009; Jerkovic et al., 2009). Linalool has cytotoxic activity over fungus and several bacteria, which could be related to the

Table 4. Ketone, terpene, aldehyde and ester composition (µg/L) of flower nectar and five Chilean unifloral honey samples of the same botanical origin (*Quillaja saponaria*) obtained via SPME-GC/MS.
Tabla 4. Composición de cetonas, terpenos, aldehídos y ésteres obtenidos mediante SPME-GC/MS de néctar de flores y cinco mieles monoflorales chilenas del mismo origen botánico (*Quillaja saponaria*).

	Nectar (µg/L)	Honey 1 (µg/L)	Honey 2 (µg/L)	Honey 3 (µg/L)	Honey 4 (µg/L)	Honey 5 (µg/L)
Ketones						
2,3-Butanedione	ND	ND	7	5	ND	ND
Isoacetophorona	ND	10	ND	ND	ND	ND
Ketoisophorona	ND	13	13	3	2.6	15.9
Acetal	ND	ND	ND	ND	ND	0.2
2-Pentanona	ND	ND	ND	ND	ND	0.5
5-Methyl-4-octanone	ND	ND	ND	ND	1.5	ND
3,5,5-Trimethyl-1,4-cyclohexanedione	ND	ND	ND	ND	ND	9.3
Hexanal dimetil acetal	27.7	ND	ND	ND	ND	ND
6-Metil-5-hepten-2-one	5.5	ND	ND	ND	ND	ND
Octanal dimetil acetal	8.4	ND	ND	ND	ND	ND
Nonanal dimetil acetal	7.5	ND	ND	ND	ND	ND
Total	49.1	23	20	8	4.2	25.9
Terpenes						
Linalool oxide 1 (Z ó E)	ND	6	12	10	13.1	13.3
Linalool oxide 2 (Z ó E)	ND	4	7	3	6.3	7
Epoxy Linalool 1 (Z ó E)	ND	3	6	5	ND	ND
Epoxy Linalool 2 (Z ó E)	ND	1	2	3	ND	ND
Epoxy Linalool isomer 1	ND	ND	ND	ND	2.2	4.9
Epoxy Linalool isomer 2	ND	ND	ND	ND	1.1	2.5
Linalool	ND	4	3	5	2.4	13.8
Ho-trienol	ND	ND	ND	2	1.6	1.4
2,6-Dimethyl-3,7-Octadiene-2,6-diol	ND	ND	ND	ND	ND	1.5
β-Pinene	3.4	ND	ND	ND	ND	ND
Limonene	44.5	ND	ND	ND	ND	ND
1,8-Cyneole (Eucaliptol)	5.8	ND	ND	ND	ND	ND
Cymene 1 (o,m ó p)	14	ND	ND	ND	ND	ND
Cymene 2 (o,m ó p)	5.2	ND	ND	ND	ND	ND
Thujone	7.7	ND	ND	ND	ND	ND
Total	80.5	18	30	28	26.7	44.3
Aldehydes						
Nonanal	3.8	3	25	8	ND	3.2
Benzaldehyde	9.6	ND	3	1	0.3	1
Octanal	ND	ND	ND	ND	ND	2.1
Phenyl acetaldehyde	ND	ND	ND	ND	5.6	12
Total	13.3	3	28	9	5.9	18.3

Ester						
Ethyl butyrate	ND	ND	ND	ND	ND	2.2
Ethyl acetate	88.5	ND	ND	ND	ND	ND
Trimethyl borate	54.5	ND	ND	ND	ND	ND
Buthyl acetate	16.7	ND	ND	ND	ND	ND
Methyl laurate	9.3	ND	ND	ND	ND	ND
Methyl caproate	5.9	ND	ND	ND	ND	ND
Etil hexanoato	3.3	ND	ND	ND	ND	ND
Methyl octanoate	4.3	ND	ND	ND	ND	ND
Methyl benzoate	6.5	ND	ND	ND	ND	ND
Total	189					

ND: Not detected

Table 5. Norisoprenoid, lactone, sulphide, furan and other composition (µg/L) of flower nectar and five Chilean unifloral honey samples of the same botanical origin (Quillaja saponaria) obtained via SPEME-GC/MS.
Tabla 5. Composición de norisoprenoides, lactonas, sulfidos, furanos y otros obtenidos mediante SPME-GC/MS de néctar de flores y cinco mieles monoflorales chilenas del mismo origen botánico (Quillaja saponaria).

	Nectar (µg/L)	**Honey 1 (µg/L)**	**Honey 2 (µg/L)**	**Honey 3 (µg/L)**	**Honey 4 (µg/L)**	**Honey 5 (µg/L)**
Norisoprenoids						
β-Damascenone	ND	4	4	12	4.3	3.4
Lactones						
Pantolactone	ND	2	3	4	3.9	7.5
Sulphides						
Dimethyl disulfide	ND	ND	ND	ND	ND	1.7
Furans						
Furfural	ND	7	31	19	17	44.2
Furfuryl alcohol	ND	ND	9	2	0.6	1.2
Total		7	39	22	17.6	45.5
Others						
3,5,5-Trimethyl-1,4-cyclohexadione	ND	4	4	ND	ND	ND

ND: Not detected.

antibacterial activity of this honey (Bakkali et al., 2008; Montenegro et al., 2009). Benzyl alcohol has been described in citrus, cotton, Christ thorn and false acacia honey (Alissandrakis et al., 2005b; Castro-Vásquez et al., 2007; Jerkovic et al., 2007; Jerkovic et al., 2009). Pantolactone has been found in chestnut honey and eucalyptus extracts, and honey from Spain (Castro-Vázquez et al., 2003; Castro-Vázquez et al., 2010). In the work of Castro-Vázquez et al. (2010), this compound was described as having a woody and toasty caramel taste.

Honey sample N°5 had higher contents of furfural and 2-phenylethanol (Tables 3 and 5) than the rest of the samples. Furans were detected in a significantly higher concentration in two out of the five honey samples analyzed (Table 5). Furan derivatives have been described as indicators of heat treatments during extraction and storage conditions. Since all honey used was harvested in the same season, these results might indicate that honey samples N°2 and N°5 were exposed to high temperatures during the extraction process and/or storage, which is congruent with the HMF content in honey N°5 (Alissandrakis et al., 2003; Piasenzotto et al., 2003). Moreover, honey N°5 presented high concentrations of 2-phenylethanol. This compound in high concentrations has been described in citrus, false acacia, Christ thorn and cambará honey (Piasenzotto et al., 2003; Alissandrakis et al., 2005a; Moreira & De Maria, 2005; Castro-Vázquez et al., 2007; Jerkovic et al., 2007; Jerkovic et al., 2009). Wani et al. (2010) reported that phenylethanol has anti-

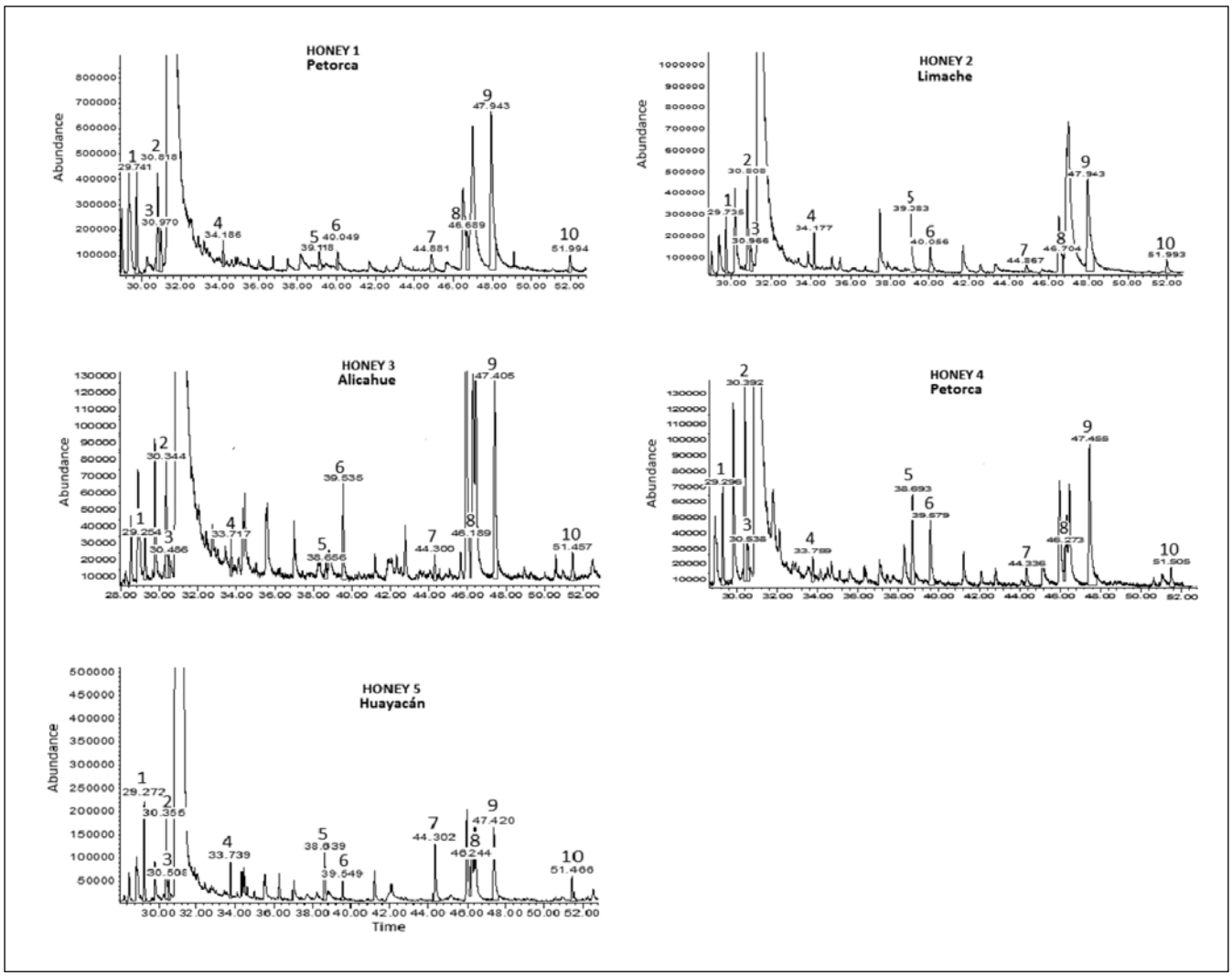

Fig. 3. GC-MS chromatogram of volatile compounds of *Quillaja saponaria* honeys. Peaks and corresponding retention time (RT): 1= linalool oxide 1 (RT: 29.254 - 29.741), 2= furfural (RT: 30.344 - 30.818), 3= linalool oxide 2 (RT: 30.486 - 30.970), 4= linalool (RT: 30.486 - 30.970), 5= 2-methyl butyric acid (RT: 38.656 - 39.118), 6= ketoisophorone (RT: 39.535 - 40.099), 7= β- damascenone (RT: 44.300 - 44.881), 8= benzyl alcohol (RT: 46.189 - 46.704), 9= 2-phenylethanol (RT: 47.405-47.943), 10= pantolactone (RT: 51.457-51.994).
Fig. 3. Cromatograma de GC-MS de los compuestos de miel de *Quillaja saponaria*. Peaks y tiempo de retención (TR): 1= óxido de linalool 1 (TR: 29,254-29,741), 2= furfural (TR: 30,344-30,818), 3= óxido de linalool 2 (TR: 30,486-30,970), 4= linalool (TR: 30,486-30,970), 5= ácido 2--metil butírico (TR: 38,656-39,118), 6= ketoisoforona (TR: 39,535-40,099), 7= β- damascenona (TR: 44,300-44,881), 8= benzil alcohol (TR: 46,189-46,704), 9= 2-feniletanol (TR: 47,405-47,943), 10= pantolactona (TR: 51,457-51,994).

septic activity and this activity may also be associated with the antibacterial activity of *Q. saponaria* honey as reported by Montenegro et al. (2009). Phenylethanol has also been described as a characteristic compound of the first stage of the fermentation process in honey. The presence of 2-phenylethanol has been associated with exposure to high temperatures during the process of extraction and/ or storage (Vidrih & Hribar, 2007). Because of this, the high concentration of these compounds in honey samples N°4 and N°5 may be attributed to the inadequate storage after their harvest.

CONCLUSION

The chemical composition of *Q. saponaria* nectar was different than the volatile compounds found in *Q. saponaria* honey, and a relationship between them could not be established. This indicates that the volatile compounds of the floral nectar should be analyzed as precursor aroma compounds present in honey, thereby establishing a relationship between both types of compounds in order to use the chemical composition of nectar to support the search for volatile compounds as chemical markers of botanical origin.

The impact of companion species on the chemical composition of *Q. saponaria* honey was demonstrated by the variable volatile compositions found in the five honey samples analyzed. Even though five unifloral honey samples shared ten volatile compounds, the variable concentration of these compounds made it difficult to establish a clear relationship between the *Q. saponaria* honey samples. Moreover, these common chemical compounds were also present in unifloral honey of other botanical origins. For this reason, a relationship between botanical origin and volatile composition could not be established. Given this, the search for chemical compounds as indicators of botanical origin in *Q. saponaria* honey should be complemented with the analysis of other types of chemical compounds present in this kind of unifloral honey.

ACKNOWLEDGEMENTS

This research was supported by Fondecyt Grant 1110808 to Gloria Montenegro.

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