© 2015
Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 14 (5): 385 - 402
ISSN 0717 7917
www.blacpma.usach.cl

Artículo Original | Original Article

Comparison of volatile compounds in *Retanilla trinervia* [Gillies & Hook] Hook & Arn honeys from central Chile

[Comparación de compuestos volátiles en mieles de *Retanilla trinervia* [Gillies & Hook] Hook & Arn de Chile Central]

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Abstract: The search for chemical markers for determining honey authenticity as a complementary tool for melissopalynological method is an important issue in the study of honeys from different botanical origins. The objective of this study was to determine the volatile compounds in tevo (*Retanilla trinervia* [Gillies & Hook] Hook & Arn [Fam. Rhamnaceae]) honey as one of the most relevant honeys from central Chile. For the identification and quantification of volatile compounds, Solid-Phase Microextraction and Gas Chromatography with Mass Spectrometry (SPME-GC-MS) was performed. A total of 28 out of 103 volatile compounds were found common to the five tevo honeys analyzed. Nevertheless, these compounds are common in unifloral honey of different floral sources from other countries. These results represent the first record in the identification of volatile compounds in tevo honey and would indicate that tevo honey does not present specific volatile compounds that allow its clear differentiation from other unifloral honey.

Keywords: SPME-GC-MS, tevo, unifloral honey

Resumen: La búsqueda de marcadores químicos para determinar la autenticidad de la miel como una herramienta complementaria al análisis melisopalinológico es un tema importante en el estudio de las mieles de diferentes orígenes botánicos. El objetivo de este estudio fue determinar los compuestos volátiles en miel de tevo (*Retanilla trinervia* [Gillies & Hook] Hook & Arn [Fam. Rhamnaceae]), una de las mieles más relevantes de Chile Central. La identificación y cuantificación de compuestos volátiles se llevó a cabo mediante Microextracción en Fase Sólida y Cromatografía de Gases con Espectrometría de Masas (SPME-CG-MS). Un total de 28 de los 103 compuestos volátiles identificados en las cinco mieles de tevo analizadas se encontraron en común para ellas. Sin embargo, estos compuestos son comunes en la miel monofloral de diferentes fuentes florales de otros países. Estos resultados representan los primeros avances en la identificación de compuestos volátiles en la miel de tevo e indicarían que la miel de tevo no presenta compuestos volátiles específicos que permitan su clara diferenciación respecto a otras mieles monoflorales.

Palabras clave: miel monofloral, SPME-GC-MS, tevo.

Recibido | Received: March 12, 2014
Aceptado | Accepted: February 8, 2015
Aceptado en versión corregida | Accepted in revised form: September 9, 2015
Publicado en línea | Published online: September 30, 2015
FONDECYT GRANT 1110808. We also thank to the beekeepers from central Chile.
Este artículo puede ser citado como / This article must be cited as: G Montenegro, F Santander, G Nunez, C Fredes. 2015. Comparison of volatile compounds in Retanilla trinervia [Gillies &Hook.] Hook. & Arn honeys from central Chile. **Bol Latinoam Caribe Plant Med Aromat** 14 (5): 385 – 402.

INTRODUCCIÓN

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). Unifloral honey has specific volatile compounds derived from its botanical origin, creating differences in its sensory properties which make identification by chemical analysis possible (Piasenzotto *et al*., 2003; Bogdanov *et al*., 2004; Manyi-Loh *et al*., 2011). Several aromatic markers have been identified in different types of honey all over the world, these analyses are commonly performed by Gas Chromatography with Mass Spectrometry (GC-MS) (Piasenzotto *et al*., 2003; Alissandrakis *et al*., 2003; Bentivenga *et al*., 2004; Alissandrakis *et al*., 2005a; Alissandrakis *et al*., 2005b; Bianchi *et al*., 2005, Moreira & De Maria, 2005; Jerkovic *et al*., 2006; Alissandrakis *et al*., 2007a; Alissandrakis *et al*., 2007b; Jerkovic *et al*., 2007; Castro-Vázquez *et al*., 2007; Daher & Gülaçar, 2008).

Chile has a great diversity of native flora that can be used by bees (*Apis mellifera* L.) for honey production (Montenegro *et al*., 2003; Montenegro *et al*., 2008; Montenegro *et al*., 2009) therefore a range of honey types with different chemical characteristics is expected. Nevertheless, scarce information exists about the volatile compounds present in Chilean native honey and information about the chemical composition of tevo (*R. trinervia* [Gillies & Hook] Hook & Arn [Fam. Rhamnaceae]) honey, one of the most relevant honeys from central Chile (Ramirez & Montenegro, 2004), has not yet been described. *Retanilla trinervia* is a woody native species widely distributed in central Chile (Montenegro *et al*., 2008) and it is used in the traditional medicine for the treatment of inflammation caused by wounds and burns (Muñoz *et al*., 1981).

The objective of this research was to compare the volatile compounds of tevo unifloral honeys collected from the same geographical origin in order to establish possible volatile compounds as chemical markers for tevo honey.

MATERIALS AND METHODS

Honey samples and pollen analysis

During the harvest season of 2012-2013, fifty-one honey samples were collected from native forests of the same geographical area (General Libertador Bernardo O'Higgins Region [33° 51' - 35° 01', VI Region]) in Central Chile. Preliminary, honey samples were studied using melissopalynological analysis to certify 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). Information about the vegetation of all the sites of sampling was also recorded.

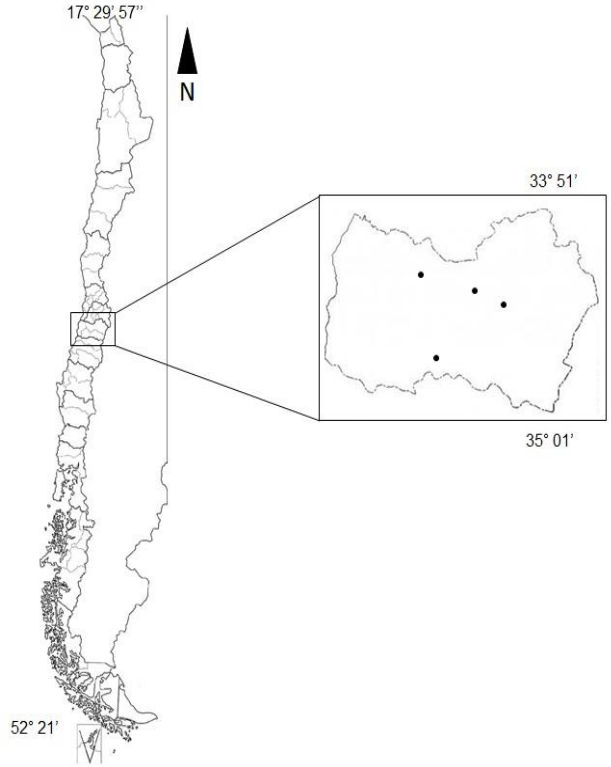


Figure 1
Map of General Libertador Bernardo O'Higgins Region, Chile. Black circles indicate the origin of the tevo honey samples

According to melissopalynological analysis, five tevo unifloral honeys that belonged to four sampling sites (Pichidegua, San Vicente, Malloa, Chépica) (Figure 1) were identified and selected for chemical analysis. The botanical origin (percentage of pollen grains of tevo), and companion species (percentage of pollen grains from other species) of tevo honeys samples are described in Table 1.

Table 1

Honey	% *R. trinervia*	Companion species*
1	77.9	*Crinodendron patagua* (11.8%).
2	45.3	*Myrceugenia* sp. (9.9%), *Lithraea caustica* (8.4%), *Trevoa quinquenervia* (6.5%), *Taraxacum* sp. (6.2%), *Schinus* sp. (4.4%), *Salix humboldtiana* (3.4%).
3	63.5	*Salix humboldtiana* (16.8%), *Brassica* sp. (8.8%).
4	55.1	*Salix humboldtiana* (26.2%), *Sisymbrium* sp. (6.0%), *Brassica* sp. (4.2%).
5	63.1	*Trevoa quiquenervia* (11.3%), *Medicago polymorpha* (4.9%), *Trifolium repens* (4.2%), *Myrceugenia* sp. (3.2%), *Taraxacum* sp. (3.1%).

Percentage of *Retanilla trinervia* presence in honey and companion species
***At least 3% presence of companion species was considered.**

Analysis by Solid Phase Microextraction and Gas Chromatography with Mass Spectrometry (SPME-GC-MS)

The five certified tevo 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 45° C in a water bath for 5 min and extracted after stirring for 1 h at the same temperature. After extraction, solid phase microextraction (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 260º C and column oven temperature was held for 5 min at 40º C, then raised 3º C/min until it reached 240º 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 ºC and 220 ºC, respectively. The spectrometric data was compared with those from NIST-EPA-NIH libraries (**http://www.nist.gov/srd/nist1a.htm**) that have more than 130,000 entries. The Kovat's retention indices were determined using a mix of n-alkane standards from C7 to C30 following the equation described by Ligor *et al.* (2014). Semiquantitative analysis was carried for the volatile compounds identified.

The repeatability of the extraction procedure was evaluated using 4-nonalol as internal standard. In Table 2 indicates the relative standard deviations (RSD) of the 28 compounds common for honey samples checked.

RESULTS

Pollen analysis (melissopalynology)

The results of botanical origin indicate that the five unifloral honey samples had a presence of tevo between 45.3 - 77.9% whereas the companion species oscillated between 22.1 - 54.3% (Table 1). Twelve different pollen grains were identified by melissopalynological analysis where native species to Chile such as *Crinodendron patagua, Lithraea caustica, Salix humboldtiana, Trevoa quinquenervia,* predominated.

Comparison of volatile compounds among tevo honeys

The RSD of the 28 common compounds in the five honey samples were found in a range of 0.5-29.1% (Table 2). Similar RSD values (below 20%) have been described by Alissandrakis *et al.* (2003) and Piasenzotto *et al.* (2003). Nevertheless, our results showed higher RSD for hexanal (Table 2).

Table 2

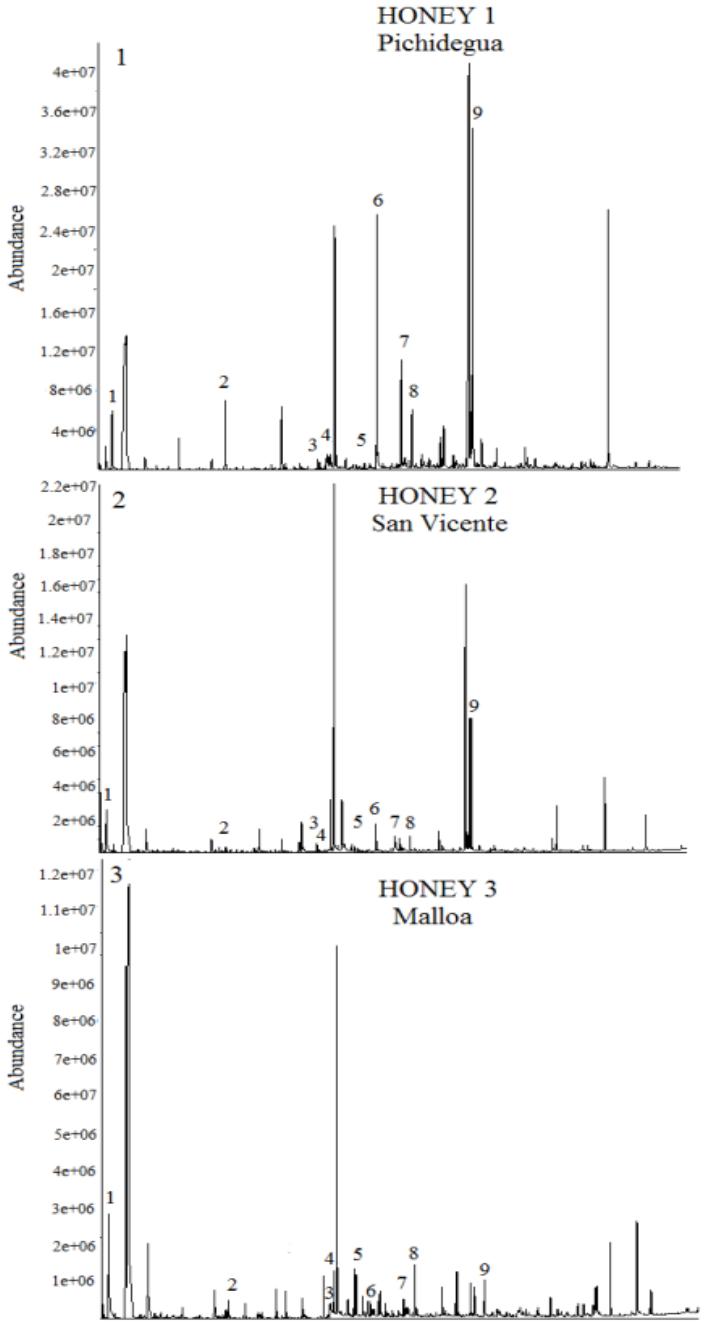
N°	Compound	%RSD
1	Acetone	14.0
2	Hexanal	29.1
3	1-Butanol	16.3
4	Heptanal	13.8
5	Limonene	11.0
6	Isoamyl alcohol	12.5
7	Ethyl hexanoate	6.2
8	o-cymene (ρ-cymene or m-cymene) Isomer 1	13.4
9	Acetoin	17.5
10	2-Heptanol	7.0
11	1-Hexanol	15.2
12	o-cymene (ρ-cymene or m-cymene) Isomer 2	9.4
13	Nonanal	17.0
14	Acetic acid	10.4
15	Furfural	17.5
16	Linalool oxide 2 (Z ó E) LOF 2	16.9
17	Benzaldehyde	5.3
18	Linalool	0.5
19	Isophorone	15.9
20	Hotrienol	0.8
21	Ethyl decanoate	14.3
22	Furfuryl alcohol	12.7
23	Ketoisophorone	17.2
24	Ethyl dodecanoate	1.9
25	Guaiacol	15.7
26	2-Phenyl etanol	13.1
27	4-Vinylguaiacol	18.2
28	Dehydro methyl jasmonate	14.7

Relative standard deviation (RSD) of 28 compounds obtained from a honey sample, estimated from 3 extractions

An example of chromatograms obtained by GC-MS of each tevo honeys and the nine volatile compounds (acetone, isoamyl alcohol, acetic acid, furfural, benzaldehyde, isophorone, furfuryl alcohol, ketoisophorone and 2-phenylethanol) with the highest mean concentration are shown in Figure 2. Kovat's retention indices were calculated in based on the mix of nalkanes standards from C7 to C30. The retention times of compounds detected in tevo honeys and calculated retention indices are presented in Table 3.

The volatile compounds identified in the five tevo honey samples differ both in type (volatile profile) and relative concentration (Table 4). In the five honey samples acetic acid was found as the only type of acid in concentrations between 1.1 - 12.7 µg/L. Among alcohols, benzyl alcohol (0.0 - 0. 7 µg/L), 2-phenylethanol (5.8 – 21.3 µg/L), 1-butanol (0.1 – 1.1 µg/L), isoamyl alcohol (0.6 – 39.0 µg/L), 1-hexanol (0.1 - 0.9 µg/L) and 2-heptanol (0.8 - 5.8 µg/L) were identified. Five ketones were identified in the five honey samples, acetone (3.8 - 61.3 µg/L), acetoin (1.5 - 6.9 µg/L), ketoisophorone (8.6 - 56.1 µg/L) and isophorone (11.5 - 316.2 µg/L). Furans were identified in the form of furfural (3.7 - 72.3 µg/L) and furfuryl alcohol (1.4 - 27.0 µg/L). Four esters were found common, ethyl hexanoate (0.3 - 1.4 µg/L), ethyl decanoate (0.6 - 3.6 µg/L), ethyl dodecanoate (0.3 - 2.3 µg/L) and dehydro methyl jasmonate (1.6 - 6.6 µg/L). Among aldehydes, hexanal (0.1 - 1.2 µg/L), heptanal (0.1 - 0.7 µg/L), nonanal (0.5 - 10.3 µg/L) and benzaldehyde (3.2 - 24.2 µg/L) were identified. Seven terpenes were found, limonene (0.4 - 1.8 µg/L), cymene 1 (0.1 - 1.0 µg/L), cymene 2 (0.1 - 0.4 µg/L), linalool oxide 2 (0.2 - 0.8 µg/L), hotrienol (0.2 - 5.4 µg/L). Among phenols two compounds were identified common for all honey samples,

guaiacol (0.7 - 5.3 µg/L) and 4-vinylguaiacol (0.3 - 1.1 µg/L).



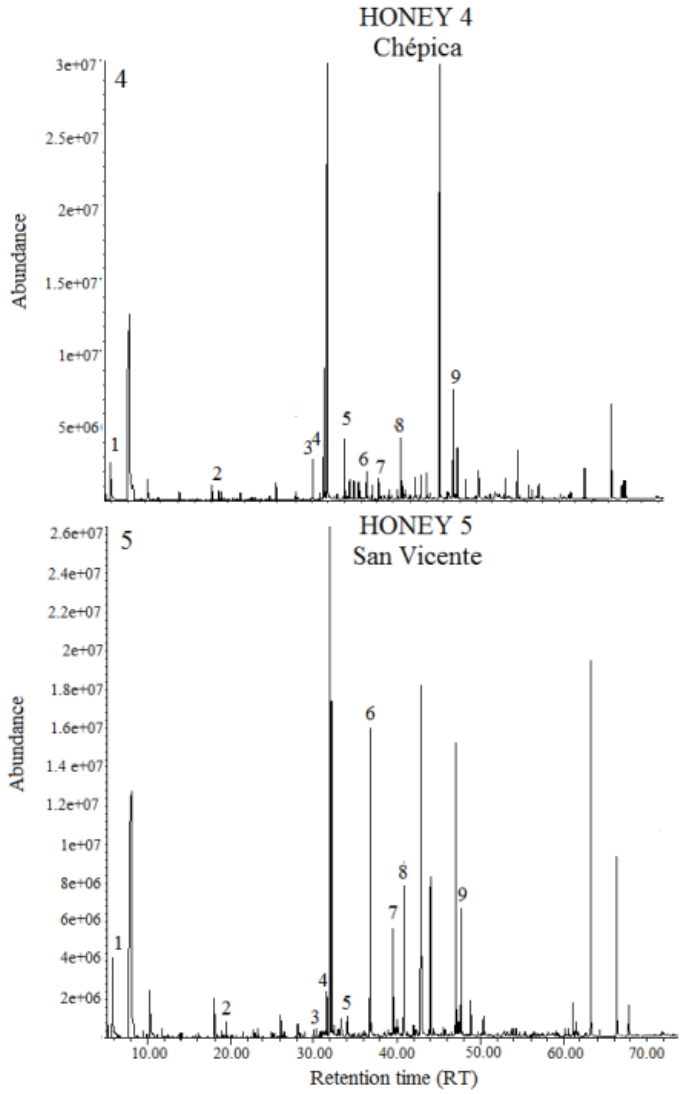


Figure 2
GC-MS chromatogram of volatile compounds of tevo honeys. Peaks and corresponding retention time (RT):
1 = acetone (R.T.: 5.22-5.23);
2 = isoamyl alcohol (R.T.: 19.52-19.54);
3 = acetic acid (R.T.:31.08-31.09);
4 = furfural (R.T.: 31.58-31.59);
5 = benzaldehyde (R.T.: 34.07-34.09);
6 = isophorone (R.T.: 36.80-36.81);
7 = furfuryl alcohol (R.T.: 39.59-39.59);
8 = ketoisophorone (R.T.:40.80-40.81);
9 = 2-phenylethanol (R.T.:48.76-48.76)

DISCUSSION

The main factor that influence honey chemical composition is the floral source, however there are other factors that also affect honey composition, such as geographical origin, honey maturity, storage conditions and harvest season (Gheldof & Engeseth, 2002; Kaskoniene & Venskutonis, 2010; Ruisinger & Schieberle, 2012; Agila & Barringer 2013; Santander *et al*., 2014,). In order to minimize the effect of these other factors which affect chemical composition of

honey, five tevo honey samples from the same geographical area (VI Region of Central Chile), harvested at the same maturity stage during the same harvest season (2012-2013) were selected and compared according to their volatile compounds profile and concentration.

According to our knowledge, the results obtained in this study are the first record in the chemical (volatile) composition of tevo honey and there are no records of the chemical composition of tevo plant. The different percentages of tevo and companion species presence in each honey may explain the different volatile compound profiles (Figure 2) and the wide range of concentration of volatile compounds (Table 4) among the five tevo honeys To be classified as a unifloral honey by melissopalynological analysis, at least 45% of the honey sample's pollen grain must come from a single species (Montenegro *et al.*, 2008) therefore other plant species (*C. patagua*, *Myrceugenia* sp., *L. caustica*, *T. quinquenervia*, *Taraxacum* sp., *Schinus* sp., *S. humboldtiana*, *Brassica* sp., *Sisymbrium* sp., *Medicago polymorpha* and *Trifolium repens*) influenced the chemical composition of tevo honeys studied (Table 1). This is particularly relevant when this kind of research is performed in wild plant communities like the native forest from Central Chile. Internationally, the nature of the honey used in similar studies makes possible to perform an easier correlation between the chemical composition and the botanical origin of honeys, because mostly cultivated species (citrus, lavender, eucalyptus, salvia, among others) have been used to obtain honey, resulting in a high percentage of the predominant species (and low percentage of companion species) and a consequent low variability of chemical composition among honeys of the same botanical origin. Our research was focused on sampling sites from native forest of VI Region of Chile. This situation meant that there were a wider variety of species where honeybees collected floral nectar, resulting in a lower percentage of the predominant species (tevo). On the other hand, the volatile composition of the plant of tevo, its floral nectar and the corresponding unifloral honey produced may be different because some compounds present in floral nectar are usually precursors of the volatile compounds found in honey (Alissandrakis *et al*., 2003; Santander *et al*., 2014) and there also exist interactions between chemical compounds and enzymes in the honey (Kaskoniene & Venskutonis, 2010).

Table 3

Compound	t_R (min)	Ret. index	Kovats retention index from the literature
Acetone	5.22	803	820 (**http://www.vcf-online.nl**)
3-Methyl-furan	6.23	848	832 (**http://www.vcf-online.nl**)
Ethyl propionate	8.69	948	950 (**www.Pherobase.com**)
Ethyl isobutyrate	8.94	957	955 (**www.Pherobase.com**)
2,3-Butanedione	9.45	976	977 (**www.Pherobase.com**)
1-Propanol	11.65	1037	1039 (**http://www.vcf-online.nl**)
Ethyl butanoate	11.62	1036	1046 (Prat *et al.*, 2014)
Ethyl-2-methyl butyrate	12.28	1051	1035 (Prat *et al.*, 2014))
Ethyl isovalerate	12.96	1067	1053 (**www.Pherobase.com**)
Butyl acetate	13.20	1072	1070 (**http://www.vcf-online.nl**)
Hexanal	13.56	1081	1078 (**http://www.vcf-online.nl**)
Isobutanol	14.23	1097	1094 (Prat *et al.*, 2014))
β-pinene	14.28	1098	1113 (**www.Pherobase.com**)
Isoamyl acetate	15.46	1123	1118 (**www.Pherobase.com**)
1-Butanol	16.54	1146	1138 (**www.Pherobase.com**)
Heptanal	18.44	1186	1183 (**http://www.vcf-online.nl**)
Limonene	18.84	1194	1212 (Högnadottir & Rousseff, 2003)
Isoamyl alcohol	19.52	1209	1206 (**http://www.vcf-online.nl**)

Ethyl hexanoate	20.78	1235	1224 (Qian & Reineccius, 2003)
3-Methyl-3-buten-1-ol	21.51	1250	1246 (**http://www.vcf-online.nl**)
1-Pentanol	21.53	1250	1249 (**http://www.vcf-online.nl**)
o-cymene (ρ-cymene or m-cymene)	22.47	1270	1267/1245 (**www.Pherobase.com**)
Acetoin	23.36	1288	1272 (Lee & Noble, 2003)
Octanal	23.45	1290	1289 (**http://www.vcf-online.nl**)
(Z)-rose oxide (or (E)-rose oxide)	24.00	1302	1338 (**www.Pherobase.com**)
2-Heptanol	24.78	1318	1318 (Nishimura, 1995)
2-Methyl-2-buten-1-ol	24.90	1321	1342 (**http://www.vcf-online.nl**)
6-Methyl-5-hepten-2-one	25.73	1339	1336 (**http://www.vcf-online.nl**)
1-Hexanol	26.32	1351	1352 (**http://www.vcf-online.nl**)
Nonanal	28.26	1392	1392 – 1388 (**http://www.vcf-online.nl**)
Ethyl octanoate	30.09	1434	1430 (Prat *et al.*, 2014))
Acetic acid	31.08	1456	1449 (Valim *et al.*, 2003)
6-Methyl-5-hepten-2-ol	31.25	1460	1465 (**http://www.vcf-online.nl**)
Furfural	31.58	1468	1458 (Lee & Noble, 2003)
Decanal	32.39	1487	1497 (**www.Pherobase.com**)
2-Acetylfuran	33.29	1507	1494 (**http://www.vcf-online.nl**)
Benzaldehyde	34.07	1527	1525 (Valim *et al.*, 2003)
Linalool	34.85	1545	1551 (Högnadottir & Rousseff, 2003)
1-Octanol	35.21	1554	1555 (**http://www.vcf-online.nl**)
Pinocarvone	35.81	1569	1545 (**www.Pherobase.com**)
2,3-Butanediol	36.03	1574	1568 (Prat *et al.*, 2014)
Isobutyric acid	35.96	1572	1561 (Prat *et al.*, 2014)
5-Methylfurfural	36.13	1577	1560 (**www.Pherobase.com**)
Isobornyl acetate	36.48	1585	1584 (**www.Pherobase.com**)
Isophorone	36.80	1593	1573 (**http://www.vcf-online.nl**)
Hotrienol	37.44	1609	1594 (**http://www.vcf-online.nl**)
γ-valerolactone	37.64	1614	1600 (**www.Pherobase.com**)
Myrtenal	38.25	1629	1633 (**http://www.vcf-online.nl**)
γ-butyrolactone	38.42	1634	1640 (**www.Pherobase.com**)
Ethyl decanoate	38.54	1637	1630 (Lee & Noble, 2003)
Phenyl acetaldehyde	38.97	1648	1642 (**www.Pherobase.com**)
Furfuryl alcohol	39.59	1663	1669 (Yanagimoto et al., 2004)
2-Methyl butyric acid	39.99	1673	1674 (Prat *et al.*, 2014)
Isovaleric acid	39.96	1673	1686 (**www.Pherobase.com**)
Diethyl succinate	40.09	1676	1678 (Prat *et al.*, 2014)
Borneol	41.02	1700	1693 (**http://www.vcf-online.nl**)
ρ-mentha-1,5-dien-8-ol	41.98	1725	1698 (**www.Pherobase.com**)
β-damascenone 2	45.53	1822	1832 (**www.Pherobase.com**)
Isopropyl dodecanoate	45.90	1833	1821 (**www.Pherobase.com**)
Hexanoic acid	46.56	1851	1849 (Prat *et al.*, 2014)
Guaiacol	47.02	1864	1857 (**http://www.vcf-online.nl**)

Benzyl alcohol	47.57	1880	1869 (**http://www.vcf-online.nl**)
2-Phenyl ethanol	48.76	1914	1907 (**http://www.vcf-online.nl**)
3,7-Dimethyl-1,5-octadien-3,7-diol	49.71	1942	1920 (**http://www.vcf-online.nl**)
β-ionone	49.63	1939	1947 (**www.Pherobase.com**)
Anisaldehyde	52.71	2032	2018 (**http://www.vcf-online.nl**)
o-cresol (ρ-cresol or m-cresol) 1	54.50	2087	2071 (**http://www.vcf-online.nl**)
o-cresol (ρ-cresol or m-cresol) 2	54.75	2095	2076 (**http://www.vcf-online.nl**)
Eugenol	57.16	2172	2168 (**http://www.vcf-online.nl**)
4-vinylguaiacol	58.08	2201	2192 (**http://www.vcf-online.nl**)
Methyl anthranilate	59.44	2247	2255 (**www.Pherobase.com**)
Dehydro methyl jasmonate	60.62	2286	2303 (Ka *et al.*, 2005)
Dihydroactinidiolide	62.51	2352	2348 (**www.Pherobase.com**)
4-vinylphenol	63.87	2399	2415 (**www.Pherobase.com**)
5-hydroxymethylfurfural	66.87	2507	2505 (**http://www.vcf-online.nl**)
Vanillin	68.73	2577	2577 (**www.Pherobase.com**)

Retention parameters (tR, calculated retention index, and Kovats retention index) obtained by GC-MS for compounds extracted from tevo honeys

Table 4

	Honey 1	**Honey 2**	**Honey 3**	**Honey 4**	**Honey 5**
Acids					
Acetic acid	12.7	6.7	8.3	1.1	8.3
Isobutyric acid	N.D.	N.D.	N.D.	N.D.	1.6
2-Methyl butyric acid	2.8	1.9	N.D.	0.8	4.2
Isovaleric acid	5.1	4.6	N.D.	1.0	9.4
Hexanoic acid	N.D.	5.9	1.0	N.D.	2.9
Total	20.7	19	9.3	2.9	26.4
Alcohols					
6-Methyl-5-hepten-2-ol	4.8	N.D.	N.D.	N.D.	20.2
Lilac alcohol Isomer 1	0.7	N.D.	N.D.	0.5	N.D.
Lilac alcohol Isomer 2	0.5	N.D.	N.D.	0.6	N.D.
Benzyl alcohol	0.4	0.1	0.4	0.0	0.7
2-Phenyl ethanol	20.1	5.8	14.9	15.2	21.3
1-Pentanol	0.7	N.D.	N.D.	N.D.	N.D.
1-Butanol	1.1	0.2	0.3	0.1	0.6
Isobutanol	25.2	3.4	0.6	N.D.	1.7
1-Propanol	2.1	N.D.	N.D.	N.D.	N.D.
Isoamyl alcohol	39.0	1.6	1.4	0.6	4.2
3-Methyl-3-buten-1-ol	N.D.	N.D.	N.D.	N.D.	1.0
2,3-Butanediol	8.7	N.D.	N.D.	0,4	N.D.
1-Octanol	0.2	0.4	N.D.	N.D.	0.3
1-Hexanol	0.9	0.3	0.2	0.1	0.7
2-Heptanol	5.8	1.5	0.9	0.8	3.6

2-Methyl-2-buten-1-ol	N.D.	N.D.	5.1	N.D.	1.7
Total	110.2	13.3	23.9	18.4	56.0
Ketones					
Acetone	4.6	61.3	6.7	3.8	9.3
2,3-Butanedione	N.D.	N.D.	1.9	N.D.	6.2
Acetoin	1.5	2.4	1.9	2.6	6.9
6-Methyl-5-hepten-2-one	0.1	0.3	N.D.	N.D.	N.D.
Ketoisophorone	32.6	8.6	12.1	26.3	56.1
Methyl acetophenone	1.9	N.D.	N.D.	0.7	N.D.
Isophorone	316.2	29.7	11.5	19.7	215.3
Total	356.9	102.3	34.1	53.1	293.8
Furans					
3-Methyl-furan	N.D.	0.3	0.1	N.D.	0.2
Furfural	3.7	39.0	10.6	72.3	21.2
5-Methylfurfural	N.D.	0.7	0.3	N.D.	0.4
2-Acetyl furan	N.D.	N.D.	N.D.	N.D.	2.7
Furfuryl alcohol	27.0	2.5	2.7	1.4	15.5
5-hydroxymethylfurfural	0.0	0.2	0.2	0.1	0.1
Total	30.8	42.6	13.8	73.8	40.0
Esters					
Ethyl propionate	1.2	N.D.	N.D.	N.D.	N.D.
Ethyl butyrate	N.D.	0.2	0.0	N.D.	0.1
Ethyl isobutyrate	0.3	N.D.	N.D.	N.D.	N.D.
Ethyl butanoate	0.3	N.D.	N.D.	0.1	N.D.
Ethyl-2-methyl butyrate	0.7	N.D.	N.D.	N.D.	N.D.
Ethyl isovalerate	1.4	N.D.	N.D.	N.D.	N.D.
Butyl acetate	N.D.	N.D.	N.D.	N.D.	0.1
Isoamyl acetate	N.D.	1.3	N.D.	N.D.	0.1
Ethyl hexanoate	1.4	1.2	0.3	1.0	0.4
Ethyl octanoate	N.D.	3.8	1.0	N.D.	1.4
Isobornyl acetate	0.1	N.D.	N.D.	0.3	N.D.
Ethyl decanoate	1.7	3.6	0.6	0.7	0.8
Diethyl succinate	5.0	1.0	N.D.	0.2	2.1
Isopropyl dodecanoate	0.4	N.D.	N.D.	N.D.	N.D.
Ethyl dodecanoate	2.3	1.1	0.6	0.3	1.0
Dehydro methyl jasmonate	4.7	4.1	1.6	6.6	3.3
1,4-Dimethylindanyl acetate	N.D.	N.D.	14.0	N.D.	31.2
Methyl anthranilate	N.D.	N.D.	2.9	N.D.	N.D.
Total	19.6	16.3	21.0	9.3	40.6
Aldehydes					
Hexanal	0.1	1.2	0.1	0.1	0.2
Heptanal	0.1	0.7	0.1	0.1	0.1

Octanal	N.D.	5.4	0.1	N.D.	0.1
Nonanal	0.6	10.3	0.7	0.7	0.5
Decanal	N.D.	18.0	0.7	N.D.	N.D.
Benzaldehyde	3.2	3.5	9.3	24.2	7.4
Phenyl acetaldehyde	N.D.	50.8	7.2	N.D	12.5
Lilac aldehyde Isomer 1	N.D.	0.0	1.3	1.8	0.2
Lilac aldehyde Isomer 2	N.D.	N.D.	1.3	1.7	0.2
Lilac aldehyde Isomer 3	N.D.	N.D.	1.2	1.2	0.1
Lilac aldehyde Isomer 4	N.D.	N.D.	1.0	1.3	0.1
Anisaldehyde	N.D.	N.D.	N.D.	N.D.	0.6
Hexyl cinnamic aldehyde	0.2	0.2	N.D.	0.5	N.D.
Total	4.2	90.0	23.0	31.7	22.0
Terpenes					
β-pinene	N.D.	N.D.	N.D.	N.D.	0.1
Limonene	0.4	1.6	0.6	1.8	1.6
Herboxi second isomer	N.D.	N.D.	0.4	N.D.	N.D.
o-cymene (ρ-cymene or m-cymene) Isomer 1	0.5	0.3	0.3	1.0	0.5
(Z)-rose oxide (or (E)-rose oxide)	0.2	N.D.	N.D.	N.D.	N.D.
o-cymene (ρ-cymene or m-cymene) Isomer 2	0.1	0.2	0.1	0.1	0.4
3-Hexen-1-ol (Z ó E)	0.2	0.1	0.2	N.D.	0.3
(Z)-linalool oxide (or (E)-linalool oxide Isomer 1	0.0	1.1	6.2	10.6	1.8
(Z)-linalool oxide (or (E)-linalool oxide Isomer 2	4.3	0.2	1.5	2.6	0.6
Linalool	0.5	0.2	0.8	0.6	0.6
Pinocarvone	N.D.	N.D.	0.9	N.D.	0.2
Hotrienol	3.4	0.2	3.5	5.4	0.6
Borneol	N.D.	N.D.	2.3	N.D.	1.1
3,7-Dimethyl-1,5-octadien-3,7-diol	N.D.	N.D.	0.4	N.D.	0.4
ρ-menth-1-en-9-ol	N.D.	N.D.	0.1	N.D.	N.D.
Myrtenal	N.D.	N.D.	1.2	N.D.	0.1
ρ-mentha-1,5-dien-8-ol	N.D.	N.D.	N.D.	N.D.	0.6
β-ionone	0.2	0.1	N.D.	0.0	N.D.
Iridomyrmecin	N.D.	4.5	0.9	N.D.	0.2
Thymol/carvacrol	N.D.	31.8	0.2	N.D.	0.6
Total	9.8	40.3	19.6	22.1	9.6
Lactones					
γ-Butyrolactone	N.D.	N.D.	0.9	N.D.	0.4
γ-Valerolactone	N.D.	N.D.	N.D.	N.D.	0.6
γ-vinyl-γ-valerolactone	N.D.	N.D.	1.2	N.D.	N.D.
Pantolactone	N.D.	3.2	1.6	N.D.	2.7
Total	N.D.	3.2	3.6	N.D.	3.7
Norisoprenoides					
β-Damascenone Isomer 1	6.9	0.4	0.9	10.1	N.D.

β-Damascenone Isomer 2	N.D.	N.D.	17.0	196.0	3.4
3-Hydroxy-β-damascone (DAML)	N.D.	N.D.	1.5	1.4	0.0
Dihydroactinidiolide	N.D.	0.2	N.D.	N.D.	0.3
Total	6.9	0.6	19.4	207.5	3.7
Phenols Compounds					
Guaiacol	5.3	1.4	0.7	0.7	4.1
Phenol	0.6	1.3	N.D.	0.8	1.7
o-cresol (ρ-cresol or m-cresol) Isomer 1	N.D.	0.2	0.2	N.D.	0.2
o-cresol (ρ-cresol or m-cresol) Isomer 2	N.D.	0.4	0.2	N.D.	0.3
Eugenol	0.6	N.D.	N.D.	0.2	N.D.
4-vinylguaiacol	1.1	0.8	0.3	0.3	0.9
4-vinylphenol	0.2	0.2	N.D.	0.2	N.D.
Vanillin	N.D.	0.7	N.D.	0.1	N.D.
Total	7.9	5.0	1.4	2.3	7.2
Others					
3,5,5-Trimethyl-1,4-ciclohexadione	N.D.	N.D.	N.D.	6.3	33.7
3,5,5-Trimethyl-4-hydroxy-1-cyclohexanone-2-ene	N.D.	N.D.	1.4	N.D.	9.8
Total	N.D.	N.D.	1.4	6.3	43.4

Volatile composition (µg/L) of five tevo honey samples obtained by SPME-GC-MS
N.D.: not detected under the conditions of this study

Table 5

Compound	Unifloral honey	Reference
Acetone	Orange, eucalyptus, rosemary, lavender, thyme, strawberry tree, chestnut, heather, lime, sunflower, rape, acacia.	Radovic *et al*., 2001 Pérez *et al*., 2002 Bianchi *et al*., 2005
Hexanal	Strawberry tree, lavender, eucalyptus, chestnut, lemon, orange, heather, lime, sunflower, rape, rosemary, acacia.	Radovic *et al*., 2001 Bianchi *et al*., 2005 Castro-Vázquez *et al*., 2009 Castro-Vázquez *et al*., 2010 Escriche *et al.*, 2011
1-Butanol	Strawberry tree, chestnut, heather, lime, sunflower, eucalyptus, rape, lavender, rosemary, acacia.	Radovic *et al*., 2001 Bianchi *et al.*, 2005
Heptanal	Citrus, strawberry tree, lavender, eucalyptus, garland thorn, chestnut, lime, rape, rosemary, acacia.	Radovic *et al*., 2001 Alissandrakis *et al*., 2003 Bianchi *et al*., 2005 Alissandrakis *et al.*, 2007a Alissandrakis *et al.*, 2007b Castro-Vázquez *et al*., 2009 Jerkovic *et al*., 2009
Limonene	Citrus, lemon, orange.	Alisandrakis *et al*., 2003 Alisandrakis *et al*., 2005a Alisandrakis *et al*., 2007a Alisandrakis *et al*., 2007b Escriche *et al*., 2011

Isoamyl alcohol	Willow	Jerkovic *et al*., 2014.
Ethyl hexanoate	Coriander	Jerkovic *et al*., 2013
Cymene Isomer 1	Citrus	Alissandrakis *et al*., 2007a.
Acetoin	Dandelion	Piazenssotto *et al*., 2003.
2-Heptanol	Rosemary	Castro-Vázquez *et al*., 2003.
1-Hexanol	Rosemary, lavender, thyme, chestnut, heather, lime.	Radovic *et al*., 2001 Pérez *et al*., 2002 Castro-Vázquez *et al*., 2003 Castro-Vázquez *et al*., 2010
Cymene Isomer 2	Citrus	Alissandrakis *et al*., 2007a
Nonanal	Rosemary, pine, citrus, cotton strawberry tree, heather, chestnut, garland thorn, eucalyptus, lime tree, thyme, lime, sunflower, rape, lavender, acacia, lemon, orange, coriander.	Radovic *et al*., 2001 Piazenssotto *et al*., 2003 Castro-Vázquez *et al*., 2003 Alissandrakis *et al*., 2003 Alisandrakis *et al*., 2005a Alisandrakis *et al*., 2005b Bianchi *et al*., 2005 Castro-Vázquez *et al*., 2006 Castro-Vázquez *et al*., 2007 Alisandrakis *et al*., 2007a Alisandrakis *et al*., 2007b Castro-Vázquez *et al*., 2009 Jerkovic *et al*., 2009 Castro-Vázquez *et al*., 2010 Eraslan *et al*., 2010 Escriche *et al*., 2011 Jerkovic *et al*., 2013 Escriche *et al*., 2011
Acetic acid	Rosemary, orange, eucalyptus, lavender, thyme, citrus, heather, chestnut, sage, garland thorn, buckwheat, lemon.	Pérez *et al*., 2002 Alissandrakis *et al*., 2003 Castro-Vázquez *et al*., 2003 Wolski *et al*., 2006 Jerkovic *et al*., 2006 Castro-Vázquez *et al*., 2007 Jerkovic *et al*., 2009 Castro-Vázquez *et al*., 2009 Castro-Vázquez *et al*., 2010 Escriche *et al*., 2011
Furfural	Rosemary, cotton, citrus, strawberry tree, rosemary, lavender, thyme, eucalyptus, heather, chestnut, garland thorn, dandelion, lime, sunflower, rape, acacia orange blossom, buckwheat, lemon.	Radovic *et al*., 2001 Piazenssotto *et al*., 2003 Soria *et al*., 2003 Castro-Vázquez *et al*., 2003 Alissandrakis *et al*., 2005a Alissandrakis *et al*., 2005b Bianchi *et al*., 2005 Wolski *et al*., 2006 Castro-Vázquez *et al*., 2006 Alissandrakis *et al*., 2007a Alissandrakis *et al*., 2007b Castro-Vázquez *et al*., 2007

		Jerkovic *et al*., 2009 Castro-Vázquez *et al*., 2009 Castro-Vázquez *et al*., 2010 Escriche *et al*., 2011
Linalool oxide 2	Rosemary, citrus, lavender, eucalyptus, chestnut, garland thorn, orange blossom, heather, lavender, willow.	Soria *et al*. 2003 Castro-Vázquez *et al*., 2003 Castro-Vázquez *et al*., 2007 Castro-Vázquez *et al*., 2009 Jerkovic *et al*., 2009 Castro-Vázquez *et al*., 2010 Jerkovic *et al*., 2014
Benzaldehyde	Pine, orange, eucalyptus, rosemary, lavender, thyme, citrus, cotton, strawberry tree, rosemary, heather, chestnut, sage, garland thorn, tree, cambará, lime, sunflower, rape, acacia, orange blossom, willow, lemon.	Radovic *et al*., 2001 Pérez *et al*., 2002 Piazenssotto *et al*., 2003 Soria *et al*., 2003 Alissandrakis *et al*., 2003 Alissandrakis *et al*., 2005a Alissandrakis *et al*., 2005b Bianchi *et al*., 2005 Moreira & María, 2005 Castro-Vázquez *et al*., 2006 Jerkovic *et al*., 2006 Castro-Vázquez *et al*., 2007 Alissandrakis *et al*., 2007a Alissandrakis *et al*., 2007b Castro-Vázquez *et al*., 2009 Jerkovic *et al*., 2009 Castro-Vázquez *et al*., 2010 Escriche *et al*., 2011 Silici *et al*., 2011 Jerkovic *et al*., 2014
Linalool	Rosemary, citrus, cotton, lavender, thyme, eucalyptus, chestnut, orange blossom, heather, willow, coriander.	Alissandrakis *et al*., 2003 Castro-Vázquez *et al*., 2003 Piazenssotto *et al*., 2003 Soria *et al*., 2003 Alissandrakis *et al*., 2005a Alissandrakis *et al*., 2005b Alissandrakis *et al*., 2007a Alissandrakis *et al*., 2007b Castro-Vázquez *et al*., 2007 Castro-Vázquez *et al*., 2009 Castro-Vázquez *et al*., 2010 Jerkovic *et al*., 2013 Jerkovic *et al*., 2014
Isophorone	Citrus, rosemary, thyme, eucalyptus, heather, chestnut, sage, willow, coriander.	Jerkovic *et al*., 2006 Alissandrakis *et al*., 2007a Alissandrakis *et al*., 2007b Castro-Vázquez *et al*., 2007 Castro-Vázquez *et al*., 2009 Jerkovic *et al*., 2013

		Jerkovic *et al*., 2014
Hotrienol	Rosemary, citrus, cotton, lavender, thyme, eucalyptus, heather, chestnut, garland thorn, willow, coriander.	Castro-Vázquez *et al*., 2003 Alissandrakis *et al*., 2003 Alissandrakis *et al*., 2005a Alissandrakis *et al*., 2005b Alissandrakis *et al*., 2007a Alissandrakis *et al*., 2007b Castro-Vázquez *et al*., 2009 Jerkovic *et al*., 2009 Castro-Vázquez *et al*., 2010 Jerkovic *et al*., 2013 Jerkovic *et al*., 2014
Ethyl decanoate	Rosemary, chestnut	Castro-Vázquez *et al*., 2003 Castro-Vázquez *et al*., 2010
Furfuryl alcohol	Rosemary, citrus, lavender, thyme, eucalyptus, heather, chestnut, lime, sunflower.	Radovic *et al*., 2001Castro-Vázquez *et al*., 2003 Castro-Vázquez *et al*., 2007 Castro-Vázquez *et al*., 2009
Ketoisophorone	Citrus, rosemary, lavender, thyme, eucalyptus, heather, sage; willow.	Jerkovic *et al*., 2006 Castro-Vázquez *et al*., 2007 Castro-Vázquez *et al*., 2009 Jerkovic *et al*., 2014
Ethyl dodecanoate	Chestnut	Castro-Vázquez *et al*., 2010
Guaiacol	Rosemary, chestnut, citrus, lavender, thyme, eucalyptus, heather.	Castro-Vázquez *et al*., 2003 Castro-Vázquez *et al*., 2007 Castro-Vázquez *et al*., 2009 Castro-Vázquez *et al*., 2010
2-Phenyl etanol	Heather, garland thorn, chestnut, orange, eucaliptus, rosemary, lavender, thyme, citrus, cotton, lime tree, dandelion.	Pérez *et al*., 2002 Piazenssotto *et al*., 2003 Alisandrakis *et al*., 2003 Alissandrakis *et al*., 2005a Castro-Vázquez *et al*., 2006 Castro-Vázquez *et al*., 2007 Castro-Vázquez *et al*., 2009 Jerkovic *et al*., 2009 Castro-Vázquez *et al*., 2010
4-vinylguaiacol	Linden, citrus, rosemary, lavender, thyme, eucalyptus, heather.	Blank *et al*., 1989 Castro-Vázquez *et al*., 2009
Dehydro methyl jasmonate	Pine	Eraslan *et al*., 2010 Silici *et al*., 2011

Volatile compounds found in common between tevo honeys and other unifloral honeys

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-Vázquez *et al.*, 2010; Santander *et al.*, 2014). 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; Santander *et al*., 2014) and was selected for this research. To improve volatile extraction used in a previous work (Santander *et al*., 2014) temperature of extraction was raised 10° C which resulted in enhanced %RSD values (Table 2).

As stated, 28 out of 103 volatile compounds were found in common to the five tevo honeys (Table 4) however these compounds are also present in

different honeys from several botanical origins (Table 5). For example, the nine compounds found in highest concentration (Figure 2) are found in a great number of honeys from other botanical origins. Nevertheless, there are uncommon compounds found in tevo honey, such as dehydro methyl jasmonate. This last compound has only been described in pine honey that corresponds to honeydew (not originated from floral nectar) (Eraslan *et al*., 2010; Silici, 2011) therefore further analyses are necessary to determine that this compound is specific for tevo honey.

CONCLUSION

Twenty eight volatile compounds were found in common among five tevo honeys collected from the VI Region of Central Chile. These compounds are common for other unifloral honeys from other botanical origins. Nevertheless, the results of this study represent the first record in the identification of volatile compounds in tevo honey and would indicate that under the conditions of this study, tevo honey does not present specific volatile compounds that allow its clear differentiation from other unifloral honeys.

ACKNOWLEDGMENTS

FONDECYT GRANT 1110808. We also thank to the beekeepers from central Chile.

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