

VOLATILE COMPOUNDS IN HONEY PRODUCED IN THE CENTRAL VALLEY OF ÑUBLE PROVINCE, CHILE

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ABSTRACT

Headspace solid-phase microextraction (SPME) with an 85 μm Carboxen polydimethylsiloxane (CAR/PDMS) fiber was used to extract volatile compounds, and a gas chromatograph equipped with a mass spectrometry detector (GC-MS) was used to identify the volatile compounds in honeys. Thirty-four different volatile compounds from the headspace of honey produced in the central valley of Ñuble Province, Chile, were extracted with fiber coating CAR/PDMS. The identified compounds were: 10 alcohols, 9 acids, 6 ketones, 3 aldehydes, 2 furans, 2 terpenes and 2 lactones. Only four of the volatile compounds had never been reported before as honey compounds; these being: 1,3-propanediol, 2-methyl butanoic acid, 3,4-dimethyl-3-hexen-2-one, and 6-methyl-5-octen-2-one. These four compounds were found in three of the 10 analyzed samples. The compounds found in the highest percentage of area were ethanol, acetic acid, 1-hydroxy-2-propane, 3-hydroxy-2-butane, and furfural. However, the analyzed samples did not present a distinctive profile.

Key words: solid phase microextraction, volatile compounds, honey.

INTRODUCTION

Chile's internal demand for honey has increased significantly in recent years, while exports to the main markets of the US and Europe have expanded progressively. Under these circumstances, it is necessary to produce honey with unique properties that certify its origin. The productive sector has undoubtedly demonstrated a capacity for modernization and improvement of quality and productivity by adapting to increasing consumer demand.

Like any food product, honey has unique organoleptic and aromatic properties that determine consumer preference. Aroma is one of the most important features, since it also allows detection of adulteration of the product. Aroma in honey is attributed to different low-molecular-weight chemical compounds. Some compounds, all of them volatile, are derived directly from the flowers visited by bees, therefore, the aroma has a floral origin. Others, however, are generated during honey processing and storage (Castro-Vázquez *et al.*, 2007).

Because honey inherits plants properties, its color,

aroma, flavor, density, and physical and chemical properties depend on the flowers used by bees, although weather conditions also influence honey composition and properties (Ramírez and Montenegro, 2004).

Volatile compounds in honey originate from plant components through the direct generation of aromatic compounds by bees, as well as thermo-generation of aromatic compounds and the action of microorganisms (Serra and Ventura, 2003; Bastos and Alves, 2003; Iglesias *et al.*, 2004; Castro-Vázquez *et al.*, 2006a; Baroni *et al.*, 2006).

The main volatile compounds in honey have their origins, in general terms, in different chemical families, such as: alcohols, ketones, aldehydes, acids, esters, terpenes (Zhou *et al.*, 2002; Bastos and Alves, 2003).

Several techniques have been developed to identify and qualify volatile compounds that are responsible for aroma. In solid phase micro extraction (SPME) volatile compounds are adsorbed from the sample headspace. The compounds are adsorbed by a fiber in the SPME unit. Recent studies report that SPME is efficient for the extraction of volatile compounds in honey (Pérez *et al.*, 2002; Alissandrakis *et al.*, 2007; Jerkovic *et al.*, 2009). However, the composition of volatile compounds depends on the extraction method (Alissandrakis *et al.*, 2005). Not all compounds have an impact on honey aroma since the concentration of the volatile compound must exceed the perception threshold. However, a few compounds with

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low concentrations can still contribute significantly to honey aroma (Castro-Vázquez *et al.*, 2007).

Specific volatile compounds can be considered as aroma fingerprints because they provide information about the botanic origin of the honey (Alissandrakis *et al.*, 2005; Escriche *et al.*, 2009). Several studies have been published on volatile compounds in honey (Pérez *et al.*, 2002; Castro-Vázquez *et al.*, 2006a; 2006b; Cuevas-Glory *et al.*, 2007). Nevertheless, there are no studies about the fraction of volatile compounds in honey produced in Chile. The objective of this research was to study the volatile compounds of honey produced in the central valley of Ñuble Province.

MATERIALS AND METHODS

Sample

Ten samples of honey from different apiculturists of the central valley of Ñuble Province were analyzed. The zone was divided into three sub-areas: eastern, central and western (Figure 1). The analyzed samples were from a harvest in 2007 and were stored at ambient temperature until analysis.

Procedure

A 4 g sample was placed in a 10 mL vial closed with a plastic twisted-off lid and sealed with PTFE silicone septum (Supelco, Bellefonte, Pennsylvania, USA) The sample was kept inside the vial at 30 °C in a thermo block

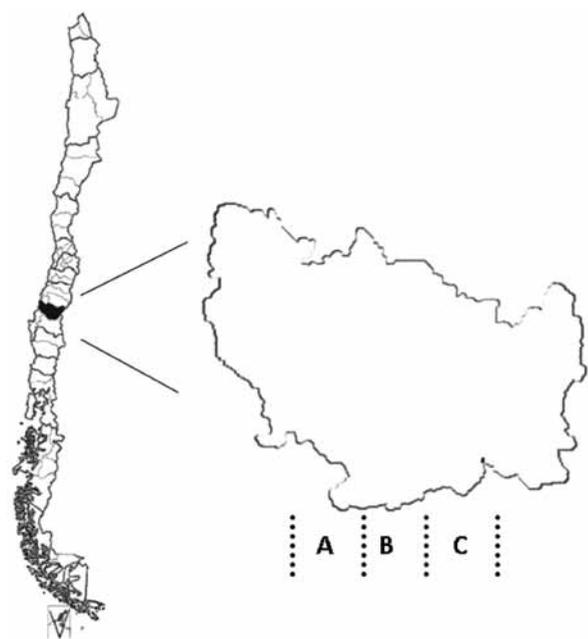


Figure 1. Sampled zones in Ñuble Province. (A) eastern; (B) central; (C) western.

(Equilab 2050-ICE, Paris, France) for 30 min to balance the headspace with the volatile compounds.

Extraction of compounds

After 30 min of balancing, the SPME fiber (Carboxen polydimethylsiloxane, CAR/PDMS 85 μm , Sigma-Aldrich, St. Louis, Missouri, USA) was exposed in the vial headspace for 1 h at 30 °C. Before analysis, fibers were preconditioned in the injection port as indicated by the manufacturer.

Identification and quantification of volatile compounds

The volatile compounds adsorbed by the fiber were desorbed in the injection port of the gas chromatograph Shimadzu GC, series GC-17-A, equipped with a mass-selective detector Shimadzu GCMS QP5050A (Kyoto, Japan). Desorption was performed by keeping the fiber in the injection port for 5 min at 220 °C with the purge valve off (splitless mode). The compounds were separated in a DB-624 capillary column 60 m long, 0.25 mm ID, with a film thickness of 1.8 μm (J&W Scientific, Folsom, California, USA). Helium was used as the carrier gas at a linear velocity of 39.7 cm s^{-1} . The temperatures of the detector and injector were 240 and 220 °C, respectively. The SPME fiber was manually injected and maintained in the injection port for 5 min, at 220 °C, with the purge valve off (splitless mode). The temperature program began when the fiber was inserted, the temperature was maintained at 38 °C for 8 min, then increased by 8 °C min^{-1} until reaching 110 °C. Subsequently, a second linear gradient of 4 °C min^{-1} was applied until reaching 220 °C. The temperature was then maintained at 220 °C for 15.5 min, for a total of 60 min. The line of transference to the mass spectrophotometer was maintained at 240 °C. Mass spectra were obtained by electron impact at 70 eV. Mass spectra data of volatile compounds were acquired in the range 25–400 amu.

Samples were randomly analyzed and results were expressed as means with standard deviation and percentage of area. Volatile compounds were identified through mass spectra as compared to library contents Nist 107 and Nist 21, and calculations were made to obtain the Kovats Index (KI) for each compound.

RESULTS AND DISCUSSION

Thirty-four volatile compounds were identified in this study of 10 samples of honey produced in different areas of the central valley of Ñuble Province: 10 alcohols, 9 acids, 6 ketones, 3 aldehydes, 2 furans, 2 terpenes and 2 lactones (Figure 2). Only four of the volatile compounds had never been reported before as honey compounds, these being: 1,3-propanodiol, 2-methyl butanoic acid,

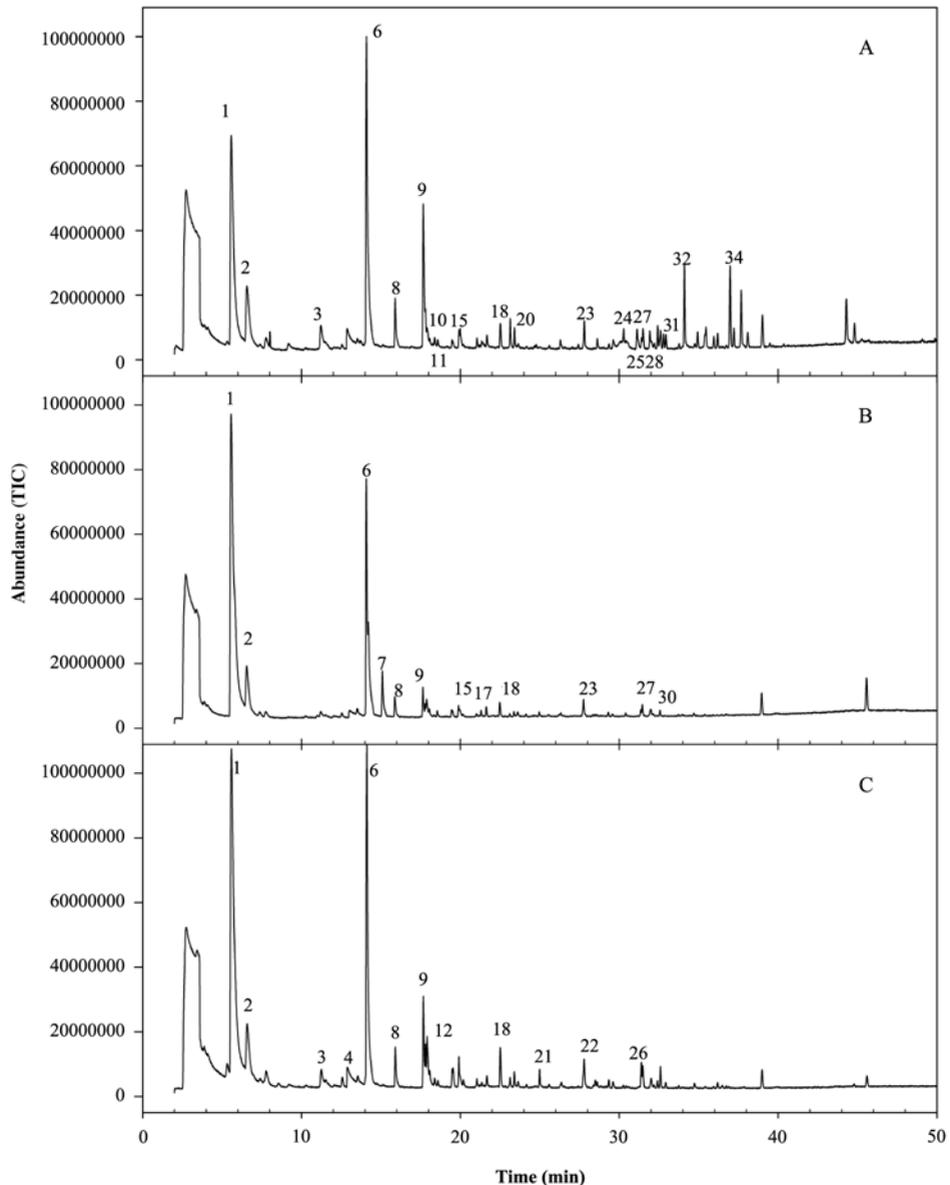


Figure 2. Chromatogram obtained by solid-phase microextraction gas-chromatography/mass spectrometry (SPME GC/MS) after maintaining the SPME fiber (85 μ m Carboxen polydimethylsiloxane) for 1 h at 30 °C in the headspace honey. (A) eastern; (B) central; (C) western. The numbers represent compounds identified and listed in Tables 1, 2 and 3.

3,4-dimethyl-3-hexen-2-one, 6-methyl-5-octen-2-one. These four compounds were found in three of the 10 analyzed samples (Tables 1, 2, and 3).

Formic acid is an organic compound found in nature. It appears in a diversity of organisms and is also one of the natural compounds of honey (Eguaras *et al.*, 2003). Two enzymatic methods, high performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE) can be used to recognize this

compound in honey (Suárez-Luque *et al.*, 2006). Mato *et al.* (2006) conducted a study to determine non-aromatic acids in honey, in which they pointed out that organic acids such as gluconic acid, formic acid and lactic acids had higher concentrations in honey than acids of botanic origins. Formic acid was identified in only two of the samples from the central valley (Tables 1 and 3).

Compounds found in all samples were: ethanol, with an area percentage between 14.9-69.21%, acetic acid with

Table 1. Volatile compound extracted after maintaining solid-phase microextraction (SPME) fiber (85 µm Carboxen polydimethylsiloxane) for 1 h at 30 °C in the headspace of eastern area honey.

N ^o a	Compound ^b	East										
		R ^c	KI ^d	Area ^e	dev. ^f	%Area ^g	Area ^e	dev. ^f	%Area ^g	Area ^e	dev. ^f	%Area ^g
1	Ethanol ^{2,5,8,11,14,15,18}	a	511	2005001638	57736513	59.97	880582044	79085349	31.64	241503696	12166997	14.19
2	Acetone ^{2,5,8,12}	a	534	52527112	17357849	1.57	258554882	78492752	9.29	292001884	62427837	17.15
3	2,3-Butanodione ^{3,12,18}	c	640	-	-	-	-	-	-	58047419	23731409	3.41
4	Formic acid	c	675	22171501	1019539	0.66	-	-	-	-	-	-
5	2-Methyl-1-propanol ^{2,5,8,18}	c	689	13230692	2716387	0.40	-	-	-	-	-	-
6	Acetic acid ^{1,2,5,10,13,14,15,16,17,18}	a	698	738635624	22703507	22.09	750418944	3136769	26.96	611172410	24229488	35.90
7	1-Butanol	b	726	-	-	-	-	-	-	37690194	1895912	2.21
8	1-Hydroxy-2-propanone ^{2,10,13,15,16,18}	c	743	35437826	2372370	1.06	79413631	3788790	2.85	102515175	4931120	6.02
9	3-Hydroxy-2-butanone ¹⁷	b	783	58839275	1674409	1.76	255205903	26416357	9.17	162278165	9159643	9.53
10	Propanoic acid ^{15,17,18}	c	786	30083314	1581039	0.90	34033105	2861449	1.22	64388140	2807834	3.78
11	2-Methyl propanoic acid ^{1,10,17,18}	b	786	25836154	5744218	0.77	22447617	587795	0.81	12756341	2727573	0.75
12	3-Methyl-3-buten-1-ol ^{2,10,12,13,18}	b	788	86600450	3197152	2.59	-	-	-	-	-	-
13	2-Methyl-1-butanol ^{1,2,15,18}	b	793	41806289	11641859	1.25	-	-	-	-	-	-
15	1,3-Propanodiol	c	841	-	-	-	31780290	331779	1.14	-	-	-
16	Butanoic acid ^{1,10,12,16,18}	b	869	18834940	8450286	0.56	-	-	-	8240742	174690	0.48
17	2,3-Butanodiol ^{1,2,15,17,18}	a	882	55098263	16674482	1.65	-	-	-	18187361	4552052	1.07
18	Furfural ^{1,2,5,6,7,8,9,10,11,13,14,15,16,17,18}	b	905	6855331	998423	0.21	37722315	1877836	1.36	38889532	5029634	2.28
19	3-Methyl butanoic acid ^{1,2,12,16,18}	b	920	-	-	-	34636483	3564504	1.24	-	-	-
20	2-Methyl butanoic acid ^{10,14}	b	931	10896222	824235	0.33	20842350	1419074	0.75	-	-	-
22	Benzaldehyde ^{1,2,4,5,6,7,8,9,10,12,13,14,15,16,17,18}	a	1034	22273241	2943934	0.67	-	-	-	-	-	-
23	Butyrolactone ^{10,13,17}	b	1039	26501712	873493	0.79	27926505	1535686	1.00	26570752	897325	1.56
24	Valerolactone ^{3,17}	c	1114	-	-	-	20404640	3080984	0.73	-	-	-
25	2-Furramethanol ^{6,7,17,18}	c	1118	51282686	10894735	1.53	29078502	2669281	1.04	11031953	1397741	0.65
26	Benzene acetaldehyde	b	1123	-	-	-	-	-	-	17020546	1502005	1.00
27	Benzyl alcohol ^{4,6,10,12,13,15,16,17,18}	c	1126	-	-	-	31780290	331779	1.14	-	-	-
28	3,4-Dimethyl-3-hexen-2-one	c	1137	-	-	-	20991844	3022628	0.75	-	-	-
29	Linalool ^{15,18}	c	1151	-	-	-	31706390	5824609	1.14	-	-	-
30	Nonanal ^{1,4,5,7,8,9,10,11,13,14,15,16,17,18}	a	1157	-	-	-	41552985	716892	1.49	-	-	-
31	Hotrienol ^{2,5,7,11,13,14,16,17,18}	c	1161	41592797	9539931	1.24	20137671	3682366	0.72	-	-	-
32	2-Ethyl-hexanoic acid ^{14,17,18}	c	1189	-	-	-	126910869	109840328	4.56	-	-	-
34	6-Methyl-5-octen-2-one	c	1286	-	-	-	27209397	3084484	0.98	-	-	-

^aNumber of peak as in Figure 2.

^bAlready reported in (1) Shimoda *et al.* (1996), (2) Pérez *et al.* (2002), (3) Zhou *et al.* (2002), (4) Guyot-Delecker *et al.* (2002), (5) Bastos and Alves (2003), (6) Serra and Ventura (2003), (7) Alissandrakis *et al.* (2005), (8) Bianchi *et al.* (2005), (9) Verzeira *et al.* (2006), (10) Baroni *et al.* (2006), (11) Castro-Vázquez *et al.* (2006b), (12) De la Fuente (2007), (13) Cuevas-Gloria *et al.* (2006b), (14) Castro-Vázquez *et al.* (2007), (15) Pontes *et al.* (2007), (16) Cui *et al.* (2008), (17) Castro-Vázquez (2008), (18) Jerkovic *et al.* (2009), (19) Escribete *et al.* (2009).

^cR, reliability of identification: a mass spectrum and retention time identical with an authentic sample; b mass spectrum and Kovats index from literature (Gianelli *et al.* (2002); Marco *et al.* (2006)); c tentative identification by mass spectrum.

^dKI, Kovats index calculated for DB-624 capillary column (J&W Scientific; 60 m, 0.32 mm ID, 1.8 µm) installed on a gas chromatograph equipped with detector, chromatographic conditions detailed in Materials and Methods.

^eResults expressed as mean of three replicates of total ion current (TIC) area of gas chromatography-mass spectrometry (GC-MS).

^fStandard deviation.

^gPercentage of TIC area.

Table 2. Volatile compound extracted after maintaining solid-phase microextraction fiber (85 µm Carboxen polydimethylsiloxane) for 1 h at 30 °C in the headspace of central area honey.

N ^o	Compound ^b	Centre										
		R ^c	KI ^d	Area ^e	dev. ^f	%Area ^g	Area ^e	dev. ^f	%Area ^g	Area ^e	dev. ^f	%Area ^g
1	Ethanol ^{2,5,8,11,14,15,18}	a	511	1366116939	212533280	69.21	559379055	485922259	33.54	832938900	144934178	31.74
2	Acetone ^{2,5,8,12}	a	534	-	-	-	130121692	82706932	7.80	252688616	158662913	9.63
5	2-Methyl-1-propanol ^{2,5,8,18}	c	689	-	-	-	-	-	-	56603174	37821600	2.16
6	Acetic acid ^{1,2,5,10,13,14,15,16,17,18}	a	698	367712005	46316049	18.63	602847909	42701995	36.15	710501803	34464633	27.07
7	1-Butanol	b	726	38093758	3251598	1.93	-	-	-	-	-	-
8	1-Hydroxy-2-propanone ^{2,10,13,15,16,18}	c	743	33742049	4287247	1.71	54617869	3508447	3.27	99933603	42919497	3.81
9	3-Hydroxy-2-butanone ¹⁷	b	783	49050153	3252115	2.48	96318282	2240531	5.78	97188756	8232345	3.70
10	Propanoic acid ^{15,17,18}	c	786	-	-	-	37827417	3902165	2.27	54820786	681090	2.09
11	2-Methyl propanoic acid ^{1,10,17,18}	b	787	-	-	-	-	-	-	28520134	14300475	1.09
12	3-Methyl-3-buten-1-ol ^{2,10,12,13,18}	b	788	35553253	2712279	1.80	71938604	2254092	4.31	137833667	23561578	5.25
14	3-Methyl-2-buten-1-ol ^{2,4,5,14}	b	829	-	-	-	-	-	-	52422335	34460462	2.00
15	1,3-Propanediol	b	840	-	-	-	-	-	-	23209498	17712205	0.88
17	2,3-Butanediol ^{1,2,15,17,18}	a	882	11456354	2061957	0.58	10109699	736020	0.61	6935109	42280	0.26
18	Furfural ^{1,2,5,6,7,8,9,10,11,13,14,15,16,17,18}	b	905	13590201	4876591	0.69	24280932	2311451	1.46	77405761	3148688	2.95
19	3-Methyl butanoic acid ^{1,2,12,16,17}	b	920	-	-	-	12846948	2752624	0.77	-	-	-
20	2-Methyl butanoic acid ^{10,14}	b	931	6178205	346117	0.31	8938574	245145	0.54	17469685	1768635	0.67
21	2-Methyl 2-butenic acid	c	965	-	-	-	-	-	-	4444741	3350502	0.17
23	Butyrolactone ^{10,13,17}	b	1039	24743590	3368284	1.25	28430222	2227014	1.70	34662214	844949	1.32
24	Valerolactone ^{13,17}	c	1114	-	-	-	-	-	-	6969079	5103954	0.27
25	2-Furanmethanol ^{6,7,17,18}	c	1118	14632028	880720	0.74	10148777	433628	0.61	41536453	3473666	1.58
26	Benzene acetaldehyde ^{1,2,9,15,18}	b	1123	8146808	158412	0.41	19954821	2801702	1.20	31776049	1943844	1.21
27	Benzyl alcohol ^{4,6,10,12,13,15,16,17,18}	c	1126	-	-	-	-	-	-	42907050	24408323	1.63
30	Nonanal ^{1,4,5,7,8,9,10,11,13,14,15,16,17,18}	a	1157	4855843	190383	0.25	-	-	-	-	-	-
33	Phenyl ethyl alcohol ^{1,9,15,18}	c	1220	-	-	-	-	-	-	13859156	8630844	0.53

^aNumber of peak as in Figure 2.

^bAlready reported in (1) Shimoda *et al.* (1996), (2) Pérez *et al.* (2002), (3) Zhou *et al.* (2002), (4) Guyot-Declercq *et al.* (2002), (5) Bastos and Alves (2003), (6) Serra and Ventura (2003), (7) Alissandrakis *et al.* (2005), (8) Bianchi *et al.* (2005), (9) Verzea *et al.* (2006), (10) Baroni *et al.* (2006), (11) Castro-Vázquez *et al.* (2006b), (12) Cuevas-Glory *et al.* (2007), (13) De la Fuente (2007), (14) Castro-Vázquez *et al.* (2007), (15) Pontes *et al.* (2007), (16) Cui *et al.* (2008), (17) Castro-Vázquez (2008), (18) Jerkovic *et al.* (2009), (19) Esriche *et al.* (2009).

^cR, reliability of identification: a mass spectrum and retention time identical with an authentic sample; b mass spectrum and Kovats index from literature (Gianelli *et al.* (2002); Marco *et al.* (2006)); c tentative identification by mass spectrum.

^dKI, Kovats index calculated for Db-624 capillary column (J&W Scientific; 60 m, 0.32 mm ID, 1.8 µm) installed on a gas chromatograph equipped with detector; chromatographic conditions detailed in Materials and Methods.

^eResults expressed as mean of three replicates of total ion current (TIC) area of gas chromatography-mass spectrometry (GC-MS).

^fStandard deviation.

^gPercentage of TIC area.

an area percentage between 17.32-36.15%, 1-hydroxy-2-propanone with an area percentage between 1.06-6.02%, 3-hydroxy-2-butanone with an area percentage between 1.63-10.30% and furfural with an area percentage between 0.21-2.96% (Tables 1, 2, and 3).

Alcohols represent an important compound in honey; methyl alcohols, such as 3-methyl-3-buten-1-ol and 2-methyl-2-buten-1-ol have been described as adding a fresh flavor, their presence is associated with different floral origins (Castro-Vázquez *et al.*, 2007). Ethanol, 2-methyl-propanol, and 3-methyl-3-buten-1-ol were described as very relevant compounds in lavender uniflorous honey (Cuevas-Glory *et al.*, 2007). However, the presence of 3-methyl-3-buten-1-ol has also been associated with rosemary uniflorous honey (De la Fuente *et al.*, 2005).

Among the identified alcohols, ethanol presented the highest response area percentage in all analyzed samples. Bastos and Alves (2003) suggest that the high content of ethanol and 2-methyl-propanol was due to the presence of yeast. However, ethanol and 2-methyl-propanol were described as inherent compounds to lavender honey (Cuevas-Glory *et al.*, 2007). On the other hand, CAR/PDMS fiber has high affinity with low-molecular-weight compounds, such as ethanol and acetic acid (Gianelli *et al.*, 2002), which explains the large amount of all these compounds in all analyzed samples. It is important to keep in mind that the composition of volatile compounds depends on the extraction method (Alissandrakis *et al.*, 2005).

Phenyl-ethyl alcohol is present in several types of honey (Shimoda *et al.*, 1996; Serra and Ventura, 2003; Alissandrakis *et al.*, 2005), however, this alcohol was identified in only one of the samples of the central valley, with an area percentage of 0.53% (Table 2).

Carboxylic acids are chemical compounds that have different aromas, ranging from spicy to rancid depending on the length of the carbon chain. Short chain acids, like acetic acid, have spicy flavors and aromas, while butanoic acid and hexanoic acids in butter are linked to rancid aroma. On the other hand, Shimoda *et al.* (1996) pointed out that butanoic acid and 3-methyl butanoic acid give haze honey (*Rhus succedanea* L.) its typical pungent smell. The hazel is a native tree from China, India, Taiwan, Japan, and Malaysia. Butanoic acid has been identified in two eastern area honey samples with an area percentage that ranged between 0.48%-0.56% and in one western area sample with an area percentage of 0.2%. 3-methyl butanoic acid was found in one eastern area sample with a 1.24% area and in one central area with a 0.67% area.

Acetic and butyric acids might be produced in bee metabolism (Bastos and Alves, 2003). However, butyric acid was not identified in any of the 10 analyzed samples.

There is no certainty of the origin of 2-ethyl-hexanoic acid, although, this compound has been identified among the volatile compounds of wine and beer (De la Fuente *et al.*, 2007).

Ketones, such as 3-hydroxy-2 butanone and acetone, are floral markers. 3-hydroxy-2 butanone has been identified as a distinctive feature of eucalyptus honey and is considered to be a floral marker for this type of honey (Pérez *et al.*, 2002; Castro-Vázquez *et al.*, 2006a; 2006b). It was also identified as a relevant compound of lime and rosemary uniflorous honey. On the other hand, acetone was identified as a relevant compound of acacia and rosemary uniflorous honey (Cuevas-Glory *et al.*, 2007).

Acetone was found in the three eastern area samples, its area percentage ranged between 1.57-17.15%. It was also found in two of the samples from the central area, with an area percentage between 7.8-9.63% and in three western area samples with an area percentage between 5.00-13.13% (Tables 1, 2, and 3).

Bastos and Alves (2003) pointed out that the acetone compound is typical of fir honey (*Abies* spp.), one of the most common introduced tree species in Chile.

Aldehydes identified in the analyzed samples were: benzaldehyde, benzalacetaldehyde and nonanal. Benzaldehyde was found in an eastern area sample, with an area percentage of 0.67% and in one western area sample, with an area percentage of 0.80%. Some derived benzene, such as benzaldehyde, benzyl alcohol and 2-phenylethanol have been reported in most European and Australian honey, with a variety of floral origins. Benzaldehyde has also been identified as a relevant compound of lavender, acacia and rosemary honey (Soria *et al.*, 2003; Cuevas-Glory *et al.*, 2007).

Volatile compounds benzaldehyde and benzene acetaldehyde have been reported as common aromatic compounds in honey samples (Shimoda *et al.*, 1996; Serra and Ventura, 2003; Alissandrakis *et al.*, 2005; Baroni *et al.*, 2006; Castro-Vázquez *et al.*, 2006a).

Benzene acetaldehyde was identified in all of the central and western area samples, their area percentage ranged between 0.41-1.21% and 0.30-1.66%, respectively. It was also identified in one eastern area sample with an area percentage of 1.00% (Tables 1, 2, and 3). Benzene acetaldehyde is considered one of the compounds that gives honey its characteristic honey aroma (Castro-Vázquez *et al.*, 2007). This compound is also relevant in lavender uniflorous honey (Cuevas-Glory *et al.*, 2007).

Benzaldehyde, benzene acetaldehyde, and 2-phenylethanol have been isolated by using different extraction methods and form parts of the aromas of different types of honey (Alissandrakis *et al.*, 2005). Some compounds derived from benzene, such as: benzaldehyde, benzyl alcohol, and phenyl ethyl alcohol, were reported

Table 3. Volatile compound extracted after maintaining solid phase microextraction fiber (85 μm Carboxen polydimethylsiloxane) for 1 h at 30 °C in the headspace of western area honey.

N ^o a	Compound ^b	West													
		R ^c	KI ^d	Area ^e	dev. f	%Area ^g	Area ^e	dev. f	%Area ^g	Area ^e	dev. f	%Area ^g	Area ^e	dev. f	%Area ^g
1	Ethanol ^{2,5,8,11,14,15,18}	a	511	1617956005	289951222	49.74	2138350914	318472581	59.46	359971887	9125984	19.43	618859804	273316403	34.98
2	Acetone ^{2,5,8,12}	a	554	176257135	142261491	5.42	-	-	-	243180425	17866187	13.13	88540056	28013241	5.00
3	2,3-Butanodione ^{3,12,18}	c	640	31897090	9076692	0.98	-	-	-	82541521	11972864	4.46	-	-	-
4	Formic acid	c	675	-	-	-	-	-	-	-	-	-	55190203	3251259	3.12
5	2-Methyl-1-propanol ^{2,5,8,18}	c	689	-	-	-	28062610	6384333	0.78	-	-	-	-	-	-
6	Acetic acid ^{1,2,5,10,13,14,15,16,17,18}	a	698	803920336	8540756	24.71	622855690	34831858	17.32	601633116	11037338	32.48	524429518	32909126	29.64
7	1-Butanol	b	726	-	-	-	455930466	24362466	12.68	21975678	4930998	1.19	-	-	-
8	1-Hydroxy-2-propanone ^{2,10,13,15,16,18}	c	743	75143722	3844268	2.31	79573048	4566494	2.21	51984993	753917	2.81	95264542	4790971	5.38
9	3-Hydroxy-2-butanone ¹⁷	b	783	131009018	22334893	4.03	58607688	3110951	1.63	190730689	16953388	10.30	55427145	6143478	3.13
10	Propanoic acid ^{15,17,18}	c	786	46649560	3281983	1.43	-	-	-	31964960	1378154	1.73	22684719	3293277	1.28
11	2-Methyl propanoic acid ^{1,10,17,18}	b	787	35596308	12714028	1.09	-	-	-	71159943	3340328	3.84	28918737	6143478	1.63
12	3-Methyl-3-buten-1-ol ^{2,10,12,13,18}	b	788	88156779	5616441	2.71	49051240	4034473	1.36	30879778	2684113	1.67	92074815	1037038	5.20
14	3-Methyl-2-buten-1-ol ^{2,4,5,14}	b	829	13686876	6148858	0.42	-	-	-	-	-	-	-	-	-
16	Butanoic acid ^{1,10,12,16,18}	b	869	-	-	-	7202530	2052792	0.20	-	-	-	-	-	-
17	2,3-Butanodiol ^{1,2,15,17,18}	a	882	13200407	2537924	0.41	15471079	1677643	0.43	-	-	-	27952634	1217024	1.58
18	Furfural ^{2,5,6,7,9,10,11,13,14,15,16,17,18}	b	905	52504381	7180219	1.61	53111399	2577526	1.48	18228049	2784931	0.98	52438433	5755151	2.96
19	3-Methyl butanoic acid ^{1,2,12,16,18}	b	920	13357610	4457208	0.41	-	-	-	-	-	-	18572420	2451263	1.05
20	2-Methyl butanoic acid ^{10,14}	b	931	-	-	-	24230864	2053454	0.67	7129663	14069	0.38	26467906	3536533	1.50
21	2-Methyl 2-butenic acid	c	965	18923463	5400306	0.58	-	-	-	-	-	-	-	-	-
22	Benzaldehyde ^{1,2,4,5,6,7,8,9,10,12,13,14,15,16,17,18}	a	1034	-	-	-	28822916	2007966	0.80	-	-	-	-	-	-
23	Butyrolactone ^{10,13,17}	b	1039	47737676	1737881	1.47	-	-	-	29715038	4717239	1.60	14207694	1027753	0.80
25	2-Furanmethanol ^{6,17,17,18}	c	1118	25235167	3214850	0.78	-	-	-	9714662	268363	0.52	18786842	2584608	1.06
26	Benzene acetaldehyde ^{2,9,15,18}	b	1123	35229784	1830746	1.08	10834536	1489305	0.30	7847280	273723	0.42	29283307	2707760	1.66
27	Benzyl alcohol ^{4,6,9,10,12,13,15,16,17,18}	c	1126	-	-	-	-	-	-	15110241	4438381	0.82	-	-	-
30	Nonanal ^{1,4,5,7,8,9,10,11,13,14,15,16,17,18}	a	1157	26610355	456626	0.82	-	-	-	-	-	-	-	-	-
32	2-Ethyl-hexanoic acid ^{14,17,18}	c	1189	-	-	-	-	-	-	71372744	25079991	3.85	-	-	-

^aNumber of peak as in Figure 2.

^bAlready reported in (1) Shimoda *et al.* (1996), (2) Pérez *et al.* (2002), (3) Zhou *et al.* (2002), (4) Guyot-Declercq *et al.* (2002), (5) Bastos and Alves (2003), (6) Serra and Ventura (2003), (7) Alissandrakis *et al.* (2005), (8) Bianchi *et al.* (2005), (9) Verza *et al.* (2005), (10) Baroni *et al.* (2006), (11) Castro-Vázquez *et al.* (2006b), (12) De la Fuente (2007), (13) Cueva-Glory *et al.* (2007), (14) Castro-Vázquez *et al.* (2007), (15) Pontes *et al.* (2007), (16) Cür *et al.* (2008), (17) Castro-Vázquez (2008), (18) Jerkovic *et al.* (2009), (19) Esriche *et al.* (2009).

^cR, reliability of identification: a mass spectrum and retention time identical to an authentic sample; b mass spectrum and Kovats index from literature (Gianelli *et al.* (2002); Marco *et al.* (2006)); c tentative identification by mass spectrum.

^dKI, Kovats index calculated for Db-624 capillary column (J&W Scientific; 60 m, 0.32 mm ID, 1.8 μm) installed on a gas chromatograph equipped with detector; chromatographic conditions detailed in Materials and Methods.

^eResults expressed as mean of three replicates of total ion current (TIC) area of gas chromatography-mass spectrometry (GC-MS).

^fStandard deviation.

^gPercentage of TIC area.

to be present in most European and Australian honey and in a wide variety of floral origins (Castro-Vázquez *et al.*, 2007).

Nonanal was identified in one sample per each sample areas, with an area percentage of 1.49% in the eastern area, 0.25% in the central, and 0.82% in the western (Tables 1, 2, and 3). Nonanal was also extracted by using SPME and is a relevant volatile compound in eucalyptus uniflorous honey (Cuevas-Glory *et al.*, 2007). This compound presented a floral aroma (Ponce, 2006).

On the other hand, enzymes from the reductasa group, whose origin is the bee organism, may convert benzene acetaldehyde into benzyl alcohol (Bastos and Alves, 2003). Benzaldehyde in particular has been described as a characteristic aromatic compound of chestnut honey (Serra and Ventura, 2003).

Furan derived compounds identified in the honey samples were: furfural and 2-furanmethanol. Furfural was present in all samples, but 2-furanmethanol was identified in nine of the analyzed samples, only in one western area sample it did not show up. The 2-furanmethanol area percentage ranged between 0.65-1.53% in the eastern area, between 0.61-1.58% in the central and between 0.52-1.06% in the western area (Tables 1, 2, and 3). Compounds resulting from sugar degradation, such as: furfural, furfuryl alcohol and 5-methyl-furfural, derived from furan, were found in fresh citric honey. These compounds increased with storage and the increase was greater when temperature was raised from 10 to 40 °C (Castro-Vázquez *et al.*, 2008). Compounds derived from furan are considered to be indicators of thermic processes and storage. They are not considered appropriate floral markers because they lose freshness with long periods of storage or exposure to high temperatures. In this study, 5-methyl-furfural was not identified among the compounds used to indicate deterioration (Castro-Vázquez *et al.*, 2007). On the other hand, furfural was identified as a relevant compound of lime, lavender and acacia uniflorous honey, and 2-furanmethanol (furfuryl alcohol) was identified as a relevant compound in lavender uniflorous honey (Cuevas-Glory *et al.*, 2007).

Furanic compounds are commonly generated by storage and heating (Sancho *et al.*, 1992). The mild heating experienced by samples during SPME sampling, which is recommended in order to improve the extraction yield and to reduce the equilibrium time, could be partially responsible for some of these compounds (Soria *et al.*, 2003).

Two samples from the eastern area showed the presence of 3,7-dimethyl-1,5,7-octatrien-3-ol (hotrienol) with a participation percentage that ranged between 0.72-1.24%, which adds floral and fruity qualities to honey aroma (Zhou *et al.*, 2002). Hotrienol has been found in

citric honey and in a variety of honeys of different origins, especially lavender honey (Castro-Vázquez *et al.*, 2007; Cuevas-Glory *et al.*, 2007). This compound has also been reported as typical to citric honey, where it can be found in a high proportion. The same compound has also been reported as a component of several varieties of honey and essential oil of a significant number of plants; however, hotrienol could be produced thermally. Hotrienol aroma has been described as sweet and floral (Alissandrakis *et al.*, 2003; 2007).

The main terpene found in *Eucryphia lucida* honey is 2,6-dimethyl-3,7-octadien-2,6-diol, which originates from floral nectar. Part of this diol is thermally dehydrated inside the beehive or during the process of forming hotrienol (3,7-dimethyl-1,5,7-octatrien-3-ol), an important aromatic compound detected in the analysis of the headspace of this type of honey (Alissandrakis *et al.*, 2007). Hotrienol was also reported in citric honeys, such as orange honey and lavender honey (Verzera *et al.*, 2005; Cuevas-Glory *et al.*, 2007). This type of compound adds floral and fruity aroma (Zhou *et al.*, 2002). The *Eucryphia* is a small botanic genus of native trees and large bushes in the temperate regions of South America and eastcoast Australian. Studies carried out in Chile have found this native uniflorous honey derived from *Eucryphia cordifolia* (ulmo tree) (Montenegro *et al.*, 2008).

Spanish citric honey extracts are rich in terpenes and its derivations (Castro-Vázquez *et al.*, 2007). Linalool was detected in only one eastern area sample, with an area percentage of 1.14% (Table 1). This compound was predominant in citric flowers, and it is considered that 80% of the compounds of citric honey extracts derive from linalool. Thus, the distinctive feature of citric honey is the presence of linalool and its derivations (Alissandrakis *et al.*, 2003).

Cyclic esters that make a major sensorial contribution are known as lactones, butirolactone being an example (Castro-Vázquez *et al.*, 2006a). This compound was not found in fresh citric honey extracts. However, the concentration of these compounds gradually increases with higher temperature (Castro-Vázquez *et al.*, 2008).

Some organic acids, ketones and benzenes, such as 2-hydroxy-2-propanone, butanoic acid, benzil alcohol or 2-phenyl-ethanol found in fresh honey gradually increase their concentration with higher temperature and storage (Castro-Vázquez *et al.*, 2008).

CONCLUSION

Volatile compounds identified in honeys from Ñuble Province have been reported before in other honey studies. Variations in volatile composition among analyzed honeys are shown. However, all samples present a majority of

the same chemical groups. Further studies are necessary to characterize the aroma and floral origins of honeys in Ñuble Province.

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RESUMEN

Compuestos volátiles en miel producida en el Valle Central de la Provincia de Ñuble, Chile. La extracción de compuestos volátiles desde el espacio de cabeza de mieles se realiza mediante microextracción en fase sólida (SPME), utilizando una fibra de 85 μm de Carboxen polidimetilsiloxano (CAR/PDMS), el análisis de los compuestos volátiles se realiza mediante cromatografía de gases con detector de masa (GC-MS). Un total de 34 diferentes compuestos volátiles fueron extraídos desde el espacio de cabeza de mieles provenientes del valle central de la provincia de Ñuble con la fibra de CAR/PDMS. Los compuestos identificados fueron 10 alcoholes, 9 ácidos, 6 cetonas, 3 aldehídos, 2 furanos, 2 terpenos y 2 lactonas. De los compuestos volátiles sólo tres no han sido reportados con anterioridad en mieles, estos compuestos fueron: 1,3-propanodiol, ácido 2-metil butanoico, 3,4-dimetil-3-hexen-2-ona, 6-metil-5-octen-2-ona. Estos cuatro compuestos se encontraron en sólo tres de las 10 muestras analizadas. Los compuestos que se encontraron en un mayor porcentaje de área fueron etanol, ácido acético, 1-hidroxi-2-propano, 3-hidroxi-2-butano y furfural; sin embargo, las muestras analizadas no presentan un perfil aromático característico.

Palabras clave: microextracción de fase sólida, compuestos volátiles, miel.

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