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Volatile compounds of Argentinean honeys: Correlation with floral and geographical origin

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ABSTRACT

The determination of the botanical/geographical origin of honey provides assurance of the product's quality. In the present work, honeys from different ecoregions of Argentina were analysed, and the possible link between the complete pollen profile of honey samples and their volatile composition was evaluated by multivariate statistical tools. A total of 110 volatile compounds were found and semiquantified in honey samples. Redundancy analysis showed significant correlations between the volatile profile of honeys and their production region (P = .0002). According to the present results, 3,8-*p*-menthatriene; cyclopropylidenemethylbenzene; 1,1,6-trimethyl-1,2-dihydronaphthalene; 1,2,4-trimethylbenzene; α-pinene; isopropyl 2-methylbutanoate; cymene; 2,6-dimethyl-1,6-octadiene; 3-methyloctane; 1-(1,4-dimethyl-3-cyclohexen-1-yl)ethanone; terpinolene; ethyl 2-phenylacetate; naphthalene and 7 unknown compounds could be used to classify Argentinean honeys according to their geographical origin with a prediction success of 96%. Moreover, it could be concluded that honeys with *Eucalyptus* sp., *Aristotelia chilensis* and T. *Baccharis* pollen types presented some characteristic volatile compounds which could be used as floral markers.

1. Introduction

Honey's price in the market is determined by its floral source and/or its provenance. Therefore, correct discrimination is not only important for consumers who look for a product with particular characteristics, but also for producers (Consonni & Cagliani, 2015; Patrignani et al., 2015). The traditional approach to recognize the botanical origin of honey relies on melissopalynology, which can also be used to identify its geographical origin. This determination of the botanical origin of honey is based on the relative frequencies of the pollen types of nectariferous species (primary enrichment). However there are other sources of variability, such as secondary, tertiary and quaternary enrichment. Secondary enrichment is defined as the inclusion of pollen inside the hive, while tertiary enrichment can occur during the extraction process of honey, and quaternary enrichment can come from aerial contamination (von der Ohe, Persano Oddo, Piana, Morlot, & Martin, 2004). In many cases these pollen types differ from those provided by the nectariferous resource. Thus, although the pollen spectrum of a honey will definitely indicate its geographical origin, it may not clearly indicate the primary source of the nectar (Persano Oddo, Piana, & Ricciardelli D'Albore, 2007).

Therefore, the identification of honey origin is a challenging area. Many studies have focused on the characterization of honey samples by specific chemical marker compounds. Particularly, the analysis of the volatile and semivolatile fractions of honey has been used for floral and geographical characterization (Consonni & Cagliani, 2015; Stanimirova et al., 2010). Moreover, a variety of environmental contaminants, including pesticides such as 1,2-dibromoethane, 1,4-dichlorobenzene and naphthalene, could be accurately determined in the honey volatile fraction (Tananaki, Zotou, & Thrasyvoulou, 2005).

Several components have been identified in honeys from a particular floral source. Karabagias et al. (2017) considered lilac aldehyde a key compound in the characterization of citrus honeys. According to Senyuva et al. (2009) the presence of lilac aldehyde and 2-aminoacetophenone could be considered indicators of rhododendron in Turkish honey, while *p*-anisaldehyde could be consider a marker of chestnut

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honey. Besides, Castro-Vázquez, Leon-Ruiz, Alañon, Pérez-Coello, and González-Porto (2014) indicated that lavender honey could be characterized in terms of its hexanal, heptanal, nerolidol oxide and coumarin contents. On the other hand, some volatile compounds have been used for the geographical discrimination of honey samples (Sobolev, Circi, Capitani, Ingallina, & Mannina, 2017). Escriche, Sobrino-Gregorio, Conchado, and Juan-Borrás (2017) could successfully differentiate thyme honeys from Greece, Spain, Morocco and Egypt through their volatile profile.

As can be seen, most of the studies were performed in monofloral honeys from one or different geographical origins. However, there is not general consensus about the floral markers that could distinguish one honey from another.

There are different obstacles that could influence a correct association between the floral origin of honey and its volatile profile. In the first place several factors, such as geographical origin, extraction technique and climatic conditions, might influence the composition of honey volatile compounds. Secondly, it should also be considered that monofloral honeys are never actually monofloral, and it is probable that the nectar of various flowers contributes to the volatile composition of honeys (Kaškonienė & Venskutonis, 2010). However, there is no information available about a possible link between the complete pollen profile (including minor components) of honey samples and their volatile composition.

Argentina is one of the most important producers and exporters of honey worldwide (Ministerio de Agroindustria de la Nación, 2017). Nonetheless, there is only limited information about their volatile profile (Baroni et al., 2006). In the present work, honey samples from four ecoregions of Argentina were analysed: Pampa, Parana Delta and Islands, Espinal and Patagonian Forest. The first 3 ecoregions are located in Buenos Aires province, which accounts for more than 50% of Argentine's honey production (Patrignani et al., 2015). Patagonian Forest is located in the southwest of Argentina; although it is not an important apicultural region, it has attracted much attention in recent years because it lacks human intervention and offers possibilities for a contamination-free apiculture industry (Forcone, 2008).

Although numerous studies have focused on the volatile composition of honeys, their possible correlations with minor species in the pollen profile and with the geographical origin are not yet well understood. Therefore, the objective of this study was not only to analyse the volatile composition of Argentinean honeys, but also to describe a procedure to evaluate their association with the geographical/floral origin by application of multivariate statistical techniques.

2. Material and methods

2.1. Honey collection

A total of 25 honey samples from 4 ecoregions of Argentina were collected directly from producers in 2014 (6 honey samples from Pampa, 6 samples from Parana Delta and Islands, 6 samples from Espinal, and 7 from Patagonian Forest). Samples were stored in the dark at −20 °C until analysis.

2.2. Pollen analysis

The pollen content of the samples was processed and analysed following the method described by Louveaux, Maurizio, and Vorhwhol (1978), slightly modified by Fagúndez (2016). Pollen types were identified to species whenever possible, or otherwise to genus, tribe or family ranks. In order to analyse the whole pollen profile, no discrimination or preselection of "monofloral honeys" was performed.

2.3. Volatile compound analysis

Isolation of volatile compounds was performed with a purge and trap system (O.I. Analytical, 4552, College Station, TX, USA). Briefly, 10,000 g of an accurately weight honey sample were diluted with 10 mL of water; then, 15 µL of internal standard (styrene 10 ppm in acetone) were added. Samples were heated at 40 °C and directly purged with helium gas (30 mL/min) during 30 min. The volatile compounds were collected on a sample concentrator OI Analytical 4560 (College Station, TX, USA) containing the porous polymer Tenax TM TA. Trapped sample compounds were desorbed by raising the trap temperature at 180 °C for 7 min. The isolated compounds were separated with an Agilent Model 6980 gas chromatograph, coupled with an Agilent 5973 mass detector (electron impact mass spectra 70 eV) (Agilent Technologies Inc., Santa Clara, California, USA). Separation was performed on a capillary column SGE BPX5 (30 m/0.25 mm/0.25 µm). The oven temperature was programmed at 40 °C for 5 min, then temperature was increased to 55 °C at 1 °C/min, to 120 °C at 3 °C/min, to 230 °C at 10 °C/min and to 280 °C at 20 °C/min. This temperature was held for 5 min. Helium was used as the carrier gas (flow 1 mL/min) and the injector temperature was 220 °C (Tananaki, Thrasyvoulou, Giraudel, & Montury, 2007). The isolated volatile compounds were tentatively identified (match score > 80%) by comparing the mass spectra of unknown peaks with those stored in the National Institute of Standards and Technology (NIST) and Wiley electronic libraries. Besides, the retention indices for the volatile compounds were calculated using *n*-alkane series, then the experimental retention indices were compared with the values available in the literature (Hosoglu, 2018).

The relative concentration of each compound was expressed as the relative peak area, which was calculated by dividing the peak area of the isolated volatile compounds by the peak area of the internal standard (assuming the same response factor for all the compounds) (Silva et al., 2017).

2.4. Statistical data analyses

Results were processed using multivariate techniques involving principal component analysis (PCA), redundancy analysis (RDA) and linear discriminant analysis (LDA). Prior to the analysis, data were standardized and centred.

The multivariate data analysis software CANOCO 5 (Biometris, Plant Research International, Netherlands) was used for the RDA and PCA analyses. This program is particularly recommended for datasets containing many zero values, such as data from pollen and volatile compounds in honey (Braak & Šmilauer, 2012).

Two different PCAs were performed for the whole dataset: one considering only the volatile compounds of the honey samples, and the other one considering their floral origin sorted by botanical family.

RDA is a constrained ordination analysis that displays how the response variables (in this case, the volatile composition of the samples) correlate with the explanatory variables (in this case, the geographical or the floral origin of the honeys). In order to minimize the number of predictors (explanatory variables), an interactive stepwise selection procedure was performed. Only candidate predictors with adjusted P-values lower than .05 were considered for the test. If the P value associated with the RDA was lower than .05, it could be concluded that the chosen variables were governed closely by the volatile composition. Also, the variance inflation factor (VIF) was calculated and only candidate predictors with VIF < 20, were included in the analysis (Braak & Šmilauer, 2012).

LDA provides a discriminate model according to previously-defined descriptors, and the probabilities of correct classification were estimated using validation methods. The Wilks' Lambda value was calculated; this parameter indicates whether the proposed model has a good discriminating power. LDA analysis was performed with the Infostat software package 2013 (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina).

Additionally, Jaccard index (Ji) was used to establish the similitude between production regions. Ji was calculated as Ji = c/a + (b − c);

where a = number of pollen types present in ecoregion A; b = number of pollen types present in ecoregion B; c = number of pollen types shared by the ecoregions A and B (Fagúndez, Reinoso, & Aceñolaza, 2016).

3. Results and discussion

3.1. Pollen analysis: description by multivariate techniques

Honey pollen profile depends on several factors, such as the pollen production of the parent plant, the distance from the beehive to the flower field, the weather conditions, and the honey extraction technique (Dobre, Alexe, Escuredo, & Seijo, 2013). The floral origin of the pollen in the honey samples was analysed, and a total of 125 pollen types, from 57 different families, were identified and quantified. The pollen analysis results can be found in Supplementary Material (Table S1). The families that occurred in more than 90% of the studied honey samples were: Asteraceae (found in 100% of the honey samples), Fabaceae (100% of the honey samples), Myrtaceae (96% of the honey samples), and Apiaceae (92% of the honey samples).

In order to achieve the best representation of the data, a PCA was performed considering the pollen families determined in the samples. The scatter plot of variables (which indicates the direction of each original variable) and samples (which defines the position of the original data in the new space) (Patrignani et al., 2015) can be seen in Fig. 1a and b, respectively. The first two principal components explained 51.5% of the total variance among samples. It is evident from Fig. 1 that there is a strong correlation between floral origin of the pollen in the samples and their geographical origin.

Samples from the Patagonian Forest ecoregion could be associated with the presence of Proteaceae (*Lomatia hirsuta)*, Rhamnaceae (*Condalia microphylla)*, Elaeocarpaceae (*Aristotelia chilensis)*, Rosaceae, Ranunculaceae (*Ranunculus* sp.), Saxifragaceae (*Escallonia* sp.), Zygophyllaceae (*Larrea* sp.*)* and Anacardiaceae (*Schinus* sp.) families. The majority of species from this region are native and endemic flora; this result is in agreement with Forcone (2008) who analysed honeys from Patagonia during 1995 and 2004. This author suggested that, because of the abundance of native and endemic plant species (probably related to the lack of human intervention), Patagonian honeys could be distinguished from other areas of Argentina by their pollen spectrum. In line with these findings, according to the Jaccard similarity index (Ji) species from Patagonian Forest ecoregion were very different to those of Espinal (Ji = 0.36), Pampa (Ji = 0.27), and Parana Delta and Islands (Ji = 0.24).

Previous analyses have indicated that the Parana Delta and Islands ecoregion could be well characterized by hydrophilic species (Caccavari & Fagúndez, 2010; Fagúndez, 2016). The main pollen types of the honeys analysed from this region were from the families Brassicaceae (T. *Brassica campestris)*, Polygonaceae (T. *Polygonum hydropiperoides)*, Myrtaceae (*Eucalyptus* sp.), Alismataceae (*Sagittaria montevidensis*), and Asteraceae. In line with these findings, Caccavari and Fagúndez (2010) found that Polygonaceae, Alismataceae, Asteraceae, Pontederiaceae and Salicaceae families were the main botanical families represented by pollen in honeys from the Parana Delta and Islands ecoregion.

Finally, it can be seen from Fig. 1 that the pollen spectrum from Pampa and Espinal ecoregions was very similar and the Jaccard index was the highest observed between ecoregions (Ji = 0.53). Apiaceae *(Ammi majus, Eryngium* sp.), Fabaceae (*Lotus* sp., *Trifolium repens*, *Melilotus albus)*, Lamiaceae (*Mentha pulegium),* and Myrtaceae (*Eucalyptus* sp.) were the most representative families from both regions. In this case, the only species that could be classified as native were *Eryngium* sp. and *Lycium* sp.

Agriculture and livestock production have been intensively developed in Buenos Aires province. This has had a huge impact on the native vegetation, thus native species were minimally represented in honeys from this province. Moreover, because of the geographical proximity of the production ecoregions some similarities in the pollen spectrum were found. Bees can generally forage within 1 km of the hive, but they can fly for more than 14 km if necessary to exploit other plant species (Fagúndez, 2016; Ratnieks & Shackleton, 2016). This may increase the diversity of flowers used by a colony (Fagúndez, 2016). This could lead to some mistakes in honey classification. For example, although sample P1 was classified as Pampa ecoregion, it had some minor pollen components such as *Scutia buxifolia, Celtis* sp., T. *Baccharis*, and *Schinus* sp., which are characteristic flora of the Espinal

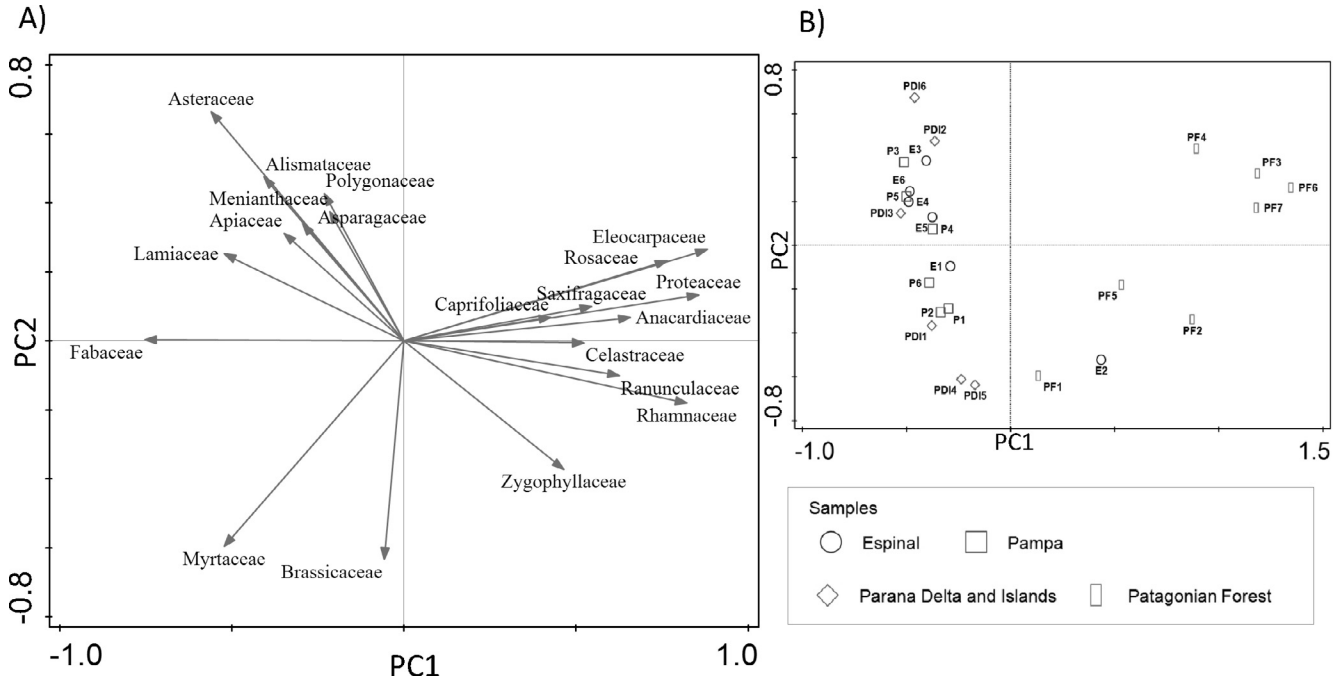

Fig. 1. PCA scatter plots of (A) pollen families found in honey samples and (B) honey samples of different ecoregions (Espinal (E), Pampa (P), Parana Delta and Islands (PDI), and Patagonian Forest (PF)).

Table 1
Volatile compounds in honey samples from four different regions of Argentina (Patagonian Forest, Espinal, Pampa and Parana Delta and Islands). Unidentified compounds were indicated as "Unknown" followed by the *m/z* values of the fragment ions (base peak underlined). Results are expressed as average values and standard deviation of the relative concentration.*

#	Name	RI	RI_{lit}	Patagonian Forest	Espinal	Pampa	Parana Delta and Islands
1	3-Methyl-2-butenal	< 800	800[a]	0.068(0.079)	0.038(0.069)	0.002(0.004)	0.037(0.054)
2	Octene	< 800	790[a]	0.137 (0.091)	0.014(0.016)	0.045(0.033)	0.007(0.017)
3	Octane	800	800[a]	1.434 (0.329)	0.703(0.198)	0.561(0.060)	0.872(0.528)
4	Furfural (IUPAC name: 2-Furaldehyde)	828	834[b]	0.396(0.197)	0.179(0.17)	0.169(0.086)	0.299(0.286)
5	Unknown 1 (57,85,128)	831	–	0.330(0.634)	Nd	0.011(0.027)	0.243(0.438)
6	Unknown 2 (90,91,107,122)	835	–	0.137(0.229)	Nd	Nd	0.003(0.008)
7	Ethyl 2-methylbutanoate	846	845[a]	0.001(0.002)	0.017(0.029)	0.0001(0.0003)	0.006(0.007)
8	Ethyl 2-methylbutanoate	849	845[a]	Nd	0.055(0.103)	Nd	0.003(0.008)
9	Unknown 3 (79,109,124)	849	–	Nd	0.056(0.103)	Nd	0.003(0.008
10	Unknown 4 (55,73)	854	–	Nd	Nd	Nd	0.035(0.058)
11	Ethylbenzene	852	852[b]	0.009(0.017)	0.004(0.006)	0.054(0.0961)	0.001(0.001)
12	*p*-Xylene	860	860[b]	0.055(0.106)	0.0266(0.035)	0.250(0.506)	0.002(0.002)
13	3-Methyloctane	865	874[a]	Nd	Nd	0.001(0.002)	0.024(0.038)
14	2-Heptanone	892	890[b]	0.051(0.058)	0.007(0.008)	0.011(0.009)	0.021(0.014)
15	2,5-Diethyltetrahydrofuran	897	902[a]	0.007(0.012)	0.091(0.088)	0.050(0.074)	Nd
16	Unknown 5 (55,71,93,111,125)	900	–	0.092(0.261)	Nd	Nd	Nd
17	Nonane	900	900[a]	0.254(0.161)	0.171(0.057)	0.152(0.039)	0.167(0.088)
18	Heptanal	903	921[a]	0.249(0.184)	0.035(0.061)	0.015(0.011)	0.0166(0.008)
19	Unknown 6 (55,67,93,111,125)	908	–	0.019(0.033)	Nd	0.001(0.001)	Nd
20	α-Pinene (IUPAC name:(5*S*)-4,6,6-Trimethylbicyclo[3.1.1]hept-3-ene)	926	921[a]	0.013(0.021)	0.018(0.020)	0.025(0.041)	0.015(0.015)
21	2,6-Dimethylphenol	930	1080[a]	0.018(0.019)	Nd	Nd	0.001(0.001)
22	Isopropyl 2-methylbutanoate	940	1002[a]	Nd	Nd	Nd	0.059(0.100)
23	Unknown 7 (55,67,82,111)	940	–	0.049(0.055)	Nd	Nd	Nd
24	Unknown 8 (57,69,74,87)	944	–	Nd	Nd	Nd	0.056(0.094)
25	Propylbenzene	945	959[a]	0.014(0.013)	0.002(0.002)	0.011(0.019)	0.003(0.004)
26	Benzaldehyde	952	960[a]	0.813(0.340)	0.459(0.325)	0.170(0.089)	0.597(0.440)
27	1-Ethyl-4-methylbenzene	958	960[a]	Nd	Nd	0.077(0.019)	Nd
28	Unknown 9 (57,71,85,105)	960	–	Nd	Nd	0.002(0.006)	0.012(0.017)
29	Oct-1-en-3-ol	981	982[a]	Nd	0.029(0.070)	Nd	Nd
30	1,2,4-Trimethylbenzene	986	988[a]	0.113(0.104)	0.093(0.013)	0.180(0.189)	0.093(0.019)
31	6-Methyl-5-hepten-2-one	988	995[a]	0.019(0.019)	0.006(0.006)	Nd	0.016(0.030)
32	2,6-Dimethyl-1,6-octadiene	990	985[a]	0.059(0.025)	0.039(0.028)	0.068(0.030)	0.253(0.253)
33	Decane	10000	10000	0.043(0.022)	0.011(0.009)	0.011(0.013)	0.005(0.007)
34	Octanal	1008	1012[a]	0.209(0.083)	0.172(0.252)	0.043(0.026)	0.073(0.020)
35	2,6-Dimethyl-1,6-octadiene	1012	985[a]	0.022(0.023)	0.044(0.059)	0.083(0.065)	0.287(0.404)
36	Cymene (IUPAC name: 1-Isopropyl-4-methylbenzene)	1039	1025[a]	Nd	Nd	Nd	0.036(0.060)
37	6-Ethyl-6-methylfulvene	1017	–	Nd	0.002(0.005)	0.022(0.0559	Nd
38	Unknown 10 (55,57,67,71)	1019	–	0.003(0.07)	Nd	Nd	Nd
39	α-Terpinene (IUPAC name:1-Isopropyl-4-methyl-1,3-cyclohexadiene)	1024	1025[a]	0.032(0.019)	0.015(0.020)	0.022(0.046)	0.050(0.074)
40	1,2,3-Trimethylbenzene	1031	1018[a]	0.026(0.029)	0.014(0.008)	0.020(0.021)	0.010(0.011)
41	1-Methoxy-4-methylbenzene	1033	1024[a]	0.027(0.023)	Nd	Nd	Nd
42	*m*-Cymene (IUPAC name: 1-Isopropyl-3-methylbenzene)	1039	1026[a]	0.101(0.071)	0.081(0.109)	0.081(0.096)	0.058(0.101)
43	Limonene (IUPAC name: 4-Isopropenyl-1-methylcyclohexene)	1045	1041[a]	0.066(0.037)	0.065(0.095)	0.024(0.006)	0.049(0.039)
44	2-Ethyl-1-hexanol	1062	1031[a]	0.033(0.041)	0.009(0.013)	Nd	Nd
45	Isophorone (IUPAC name: 3,5,5-Trimethyl-2-cyclohexen-1-one)	1072	1117[b]	0.006(0.018)	Nd	Nd	Nd
46	Phenylacetaldehyde	1075	1063[a]	0.116(0.076)	0.187(0.081)	0.151(0.0789	0.217(0.267)
47	β-Ocimene (IUPAC name: 3,7-Dimethyl-1,3,6-octatriene)	1088	1037[a]	0.022(0.014)	0.003(0.004)	0.003(0.003)	0.015(0.0171)
48	Unknown 11(55,69,91,93,111)	1091	–	0.020(0.022)	Nd	Nd	0
49	γ-Terpinene (IUPAC name: 1-Isopropyl-4-methyl-1,4-cyclohexadiene)	1098	1070[a]	0.149(0.132)	0.042(0.030)	0.024(0.041)	0.030(0.030)
50	1-Chlorooctane	1103	1044[a]	0.016(0.016)	0.004(0.004)	0.003(0.0025)	0.010(0.011)
51	Acetophenone (IUPAC name: 1-Phenylethanone)	1105	1088[a]	0.038(0.047)	0.001(0.001)	0.001(0.001)	0.003(0.003)
52	Unknown 12 (79,93,109,137,152)	1110	–	0.004(0.011)	0.001(0.003)	0.006(0.010)	0
53	Linalool oxide isomers (IUPAC name: isomers of 2-[(2*S*,5*S*)-5-ethenyl-5-methyloxolan-2-yl]propan-2-ol)	1112	1102[a]	0.222(0.315)	0.184(0.245)	0.020(0.049)	0.162(0.183)
54	Terpinolene (IUPAC name:1-Methyl-4-propan-2-ylidenecyclohexene)	1122	1088[a]	0.036(0.043)	0.006(0.012)	0.003(0.003)	0.003(0.007)
55	1,2,3,4-Tetramethylbenzene	1123	1122[a]	0.135(0.208)	0.388(0.867)	0.691(1.461)	0.160(0.073)
56	Linalool oxide isomers	1125	1102[a]	0.018(0.044)	0.002(0.005)	Nd	Nd
57	Unknown 13 (55,58,67,94)	1098	–	0.038(0.071)	0.003(0.008)	0.010(0.0139	0.020(0.237))
58	2-Nonane	1130	1100[a]	0.046(0.070)	0.013(0.008)	0.015(0.013)	0.014(0.009)
59	Unknown 14 (79,91,107,135,150)	1132	–	0.009(0.019)	Nd	Nd	0.027(0.058)
60	Linalool (IUPAC name 3,7-Dimethyl-1,6-octadien-3-ol)	1135	1110[a]	0.672(0.625)	0.098(0.115)	0.005(0.013)	0.015(0.023)
61	Ethyl heptanoate	1136	1104[a]	Nd	0.010(0.024)	Nd	Nd
62	Nonanal	1138	1115[a]	1.609(0.848)	0.783(0.780)	0.392(0.116)	0.432(0.209)
63	Rose oxide (IUPAC name: (2*R*,4*S*)-4-Methyl-2-(2-methylprop-1-enyl) oxane)	1142	1114[a]	0.007(0.011)	0.034(0.075)	0.003(0.008)	0.0429(0.094)
64	Unknown 15 (93,108,119,134,150)	1144	–	0.009(0.016)	Nd	Nd	Nd
65	Isophorone (IUPAC name: 3,5,5-Trimethyl-2-cyclohexen-1-one	1146	1120[a]	0.151(0.189)	0.018(0.014)	Nd	Nd
66	Unknown 16 (77,109,115,119)	1153	–	0.009(0.015)	0.004(0.009)	Nd	Nd
67	1,3,8-*p*-Menthatriene (IUPAC name: 1-Isopropenyl-4-methyl-1,3-cyclohexadiene)	1157	1139[a]	0.016(0.034)	0.050(0.074)	0.090(0.058)	0.094(0.068)
68	Lilac aldehyde B (IUPAC name: 2-(5-Ethenyl-5-methyloxolan-2-yl) propanal)	1163	1145[a]	0.327(0.275)	0.033(0.051)	0.050(0.056)	0.080(0.089)

(*continued on next page*)

Table 1 (*continued*)

#	Name	RI	RI_{lit}	Patagonian Forest	Espinal	Pampa	Parana Delta and Islands
69	1-(1,4-Dimethyl-3-cyclohexen-1-yl)ethanone	1166	1145[a]	0.027(0.051)	Nd	Nd	Nd
70	Cyclopropylidenemethylbenzene	1166	–	0.017(0.025)	0.034(0.034)	0.052(0.038)	0.044(0.030)
71	Lilacaldehyde A	1168	1154[a]	0.502(0.420)	0.046(0.064)	0.080(0.062)	0.106(0.115)
72	1-Ethenyl-4-methoxybenzene	1168	1158[a]	0.009(0.017)	0.054(0.090)	Nd	Nd
73	Nerol oxide (IUPAC name: 4-Methyl-2-(2-methylprop-1-enyl)-3,6-dihydro-2H-pyran)	1171	1151[a]	0.037(0.059)	0.044(0.044)	0.051(0.035)	0.023(0.021)
74	6,6-Dimethyl-2-methylenebicyclo[2.2.1]heptan-3-one	1173	1180[a]	0.074(0.189)	0.022(0.040)	0.006(0.010)	0.002(0.005)
75	Safranal (IUPAC name: 2,6,6-Trimethylcyclohexa-1,3-diene-1-carbaldehyde)	1173	1164[a]	0.082(0.130)	0.004(0.011)	0.002(0.004)	0.004(0.009)
76	Safranal isomer	1176	1164[a]	0.013(0.033)	Nd	0.002(0.003)	Nd
77	Pinocarvone (IUPAC name:6,6-Dimethyl-2-methylenebicyclo[3.1.1] heptan-3-one)	1173	1164,2[a]	0.014(0.026)	Nd	Nd	0.004(0.010)
78	Borneol (IUPAC name: 4,7,7-Trimethylbicyclo[2.2.1]heptan-3-ol)	1175	1168[a]	0.084(0.108)	0.003(0.007)	Nd	Nd
79	Lilacaldehyde C	1178	1169[a]	0.182(0.142)	0.0156(0.025)	0.017(0.02)	0.029(0.041)
80	Unknown 17 (55,79,91,107,135,150)	1179	–	Nd	0.026(0.059)	0.007(0.006)	0.0120(0.030)
81	Ethyl benzoate	1180	1171[a]	0.021(0.06)	0.137(0.305)	0.001(0.003)	0.002(0.003)
82	Naphthalene	1182	1178[a]	0.001(0.004)	0.010(0.009)	0.001(0.001)	0.0004(0.001)
83	(−)-4-Terpineol (IUPAC name: 4-Methyl-1-propan-2-ylcyclohex-3-en-1-ol)	1183	1197[a]	0.025(0.03)	Nd	Nd	Nd
84	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydro-1-benzofuran	1188	1187[a]	0.006(0.011)	0.011(0.015)	0.011(0.008)	0.0508(0.084)
85	Unknown 18 (79,122,135,150)	1190	–	Nd	0.004(0.008)	0.006(0.015)	Nd
86	Methyl salicylate (IUPAC name: Methyl 2-hydroxybenzoate)	1193	1192[a]	0.030(0.032)	0.007(0.012)	Nd	Nd
87	Myrtenal (IUPAC name: 6,6-Dimethylbicyclo[3.1.1]hept-2-ene-2-carbaldehyde)	1194	1185[a]	0.072(0.091)	0.015(0.018)	0.004(0.005)	0.013(0.011)
88	Safranal (IUPAC name: 2,6,6-Trimethyl-1,3-cyclohexadiene-1-carbaldehyde)	1197	1218[a]	0.027(0.045)	0.002(0.003)	0.001(0.002)	0.001(0.003)
89	Ethyl octanoate	1200	1200[a]	Nd	0.041(0.094)	Nd	0.001(0.002)
90	Dodecane	1200	1200	0.030(0.016)	0.0054(0.010)	0.004(0.003)	0.001(0.001)
91	Unknown 19 (67,79,91,94,122)	1199	–	Nd	0.002(0.005)	Nd	0.020(0.033)
92	α-Ionene (IUPAC name: 1,1,6-Trimethyl-1,2,3,4-tetrahydronaphthalene)	1204	1258[a]	0.002(0.07)	0.009(0.017)	0.003(0.006)	0.011(0014)
93	Decanal	1208	1209[a]	1.301(2.088)	0.242(0.103)	0.103(0.066)	0.150(0.094)
94	4,7-Dimethyl-1-benzofuran	1209	1220[a]	Nd	Nd	0.016(0.043)	0.033(0.080)
95	p-Menth-1-en-9-al (IUPAC name: 6-Isopropyl-3-methyl-2-cyclohexen-1-ol)	1174	1217[a]	0.123(0.122)	0.067(0.107)	0.069(0.077)	0.252(0.352)
96	3-Phenylfuran	1223	1227[a]	0.025(0.052)	0.003(0.005)	Nd	0.001(0.003)
97	Bornylene (IUPAC name 4,7,7-trimethylbicyclo[2.2.1]hept-2-ene)	1235	1228[c]	0.033(0.056	Nd	Nd	0.002(0.004)
98	Ethyl 2-phenylacetate	1265	1244[a]	Nd	0.079(0.160)	Nd	Nd
99	Unknown 20 (107,121,136,177,192)	1305	–	Nd	0.001(0.003)	0.001(0.001)	0.007(0.014)
100	Ethyl nonanoate	1340	1300[a]	Nd	0.023(0.056)	Nd	Nd
101	Tridecane	13000	1300	0.050(0.056)	0.031(0.023)	0.010(0.003)	0.008(0.002)
102	Undecanal	1334	1318[a]	0.021(0.023)	Nd	Nd	Nd
103	1,1,6-Trimethyl-1,2-dihydronaphthalene	1368	1359[a]	0.005(0.010)	0.021(0.029)	0.008(0.006)	0.020(0.015)
104	Unknown 21 (119,147,161,189)	1388	–	Nd	Nd	Nd	0.008(0.019)
105	β-Damascenone (IUPAC name: (2*E*)-1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one)	1395	1394[a]	0.162(0.258)	0.026(0.027)	Nd	0.003(0.004)
106	Ethyl decanoate	1409	1397[a]	Nd	0.006(0.016)	Nd	Nd
107	Unknown 22 (53.80.171)	1449	–	0.010(0.028)	Nd	Nd	Nd
108	Unknown 23 (57,71,85)	1465	–	0.017(0.034)	0.009(0.009)	Nd	Nd
109	α-Bulnesene (IUPAC name: (3*S*,3a*S*,5*R*)-5-Isopropenyl-3,8-dimethyl-1,2,3,3a,4,5,6,7-octahydroazulene)	1474	1494[a]	0.022(0.04)	Nd	Nd	0.002(0.005)
110	Pentadecane	1500	1500	0.042(0.065)	0.0110(0.012)	Nd	Nd

RI – Retention index, Lit – literature data (when available), Nd – not detected.

* The relative concentration was calculated by dividing the peak area of the isolated volatile compounds by the peak area of the internal standard styrene (10 ppm in acetone).

[a] RI obtained from http://webbook.nist.gov.

[b] RI obtained from Tananaki et al. (2007)

[c] RI obtained from Kaškonienė, Venskutonis, and Čeksterytė (2008).

ecoregion. Therefore, it could be inferred that this honey sample corresponded better to the Espinal ecoregion.

3.2. Volatile compound analysis

Volatile compounds in honey have their origin in different sources: from the plant or nectar source, derived from bee metabolism, environmental sources (contamination, fumigants), absorption of odours from the air, or they are related to honey processing and storage (Manyi-Loh, Ndip, & Clarke, 2011; Tananaki et al., 2005). Table 1 lists the 110 volatile compounds that were found and semiquantified in the honeys analysed in this work. Compounds such as octane, furfural, heptanal, 1,2,4-trimethylbenzene, 1,2,3-trimethylbenzene, 2,6-dimethyl-1,6-octadiene, phenylacetaldehyde, nonanal and tridecane were found in all samples, while compounds such as nonane, limonene and *p*-menth-1-en-9-al were identified in 95% of the samples.

Baroni et al. (2006) evaluated the volatile compounds of Buenos Aires honeys in samples harvested in previous years; these authors also found furfural in all honey samples. This observation could be attributed to the storage time of honey samples (Baroni et al., 2006), since furfural originates from de decomposition of monosaccharides or the Maillard reaction during the storage of honey (da Silva, Gauche, Gonzaga, Costa, & Fett, 2016).

On the other hand, according to some authors aliphatic fatty acids are assumed to originate from beeswax; this could explain the presence of compounds such as octane and tridecane in most of the samples

(Ampuero, Bogdanov, & Bosset, 2004).

The presence of heptanal in honeys is of particular interest, because it has been pointed out that this compound is characteristic of lavender or acacia honey (Castro-Vázquez et al., 2014; Plutowska, Chmiel, Dymerski, & Wardencki, 2011). However, these pollen types could not be quantified in the honey samples. This seems to indicate that heptanal could be related to the geographical origin rather than to the floral origin of the Argentinean honeys. According to the present results, it could be speculated that the presence of heptanal in Argentinean honeys may be associated to plants that grow in the geographical areas that were analysed in the current study.

The phenylacetaldehyde found in all honey samples has already been identified in numerous honey types, and it is considered that it has a pleasant aroma (Karabagias et al., 2017).

On the other hand, nonanal has been considered to be a distinctive compound of Eucalyptus honey (Kaškonienė & Venskutonis, 2010). This pollen type was found in a large number of samples analysed in the present work (more than 88%); hence, it could be speculated that there is a relation between the presence of *Eucalyptus* sp. pollen and nonanal.

Limonenene was found in 95% of the samples. Although this compound has been suggested to be a marker for citrus honey (Escriche, Visquert, Juan-Borrás, & Fito, 2009), other authors have found this compound in honeys from different floral origins such as pine, acacia and chestnut honey (Colucci, De Vito, Varricchio, De Cunzo, & Coccia, 2016; Tananaki et al., 2007). These results are in line with our findings and confirm that limonene can be found in a wide variety honeys from different floral precedence.

Another compound found in a large number of honey samples analysed in the present work was 2-heptanone. This compound was present in 80% of the Argentinean honey samples and it is known to be produced by the worker honey bees as a "forage marking pheromone" (Graham, Carroll, Teal, & Ellis, 2013).

As mentioned in the introduction, lilac aldehyde isomers are considered as "key compounds" in citrus honey, because they represent a high proportion of its total volatile fraction (Karabagias et al., 2017). In the present work, lilac aldehyde isomers were found in a high number of honey samples (more than 80%), but only a small proportion of pollen (1.5%) from *Citrus* sp. was found in only one sample from Parana Delta and Islands ecoregion. In line with this result, Baroni et al. (2006) also found that lilac aldehyde isomers were present at different percentages in a high proportion of samples from different floral origins (*Medicago sativa, Helianthus annuus, Melilotus*, and *Prosopis* spp.).

PCA was performed in order to achieve a linear projection of this complex dataset with 110 compounds. The scatter plot of variables (which shows the best 50% highest-weight volatile compounds in the diagram) and honey samples can be seen in Fig. 2a and b respectively. The first principal component (PC) accounts for 23.32% of the variance, while the second principal component accounts for 13.55%. Similar percentages of explained variance were found by Revell, Morris, and Manley-Harris (2014) in the first two PC of volatile compounds of New Zealand honeys. The PCA plots in the present work indicated some similarities among honeys from the same geographical origin. Fig. 2 shows that samples from the Patagonian Forest could be well represented by the PC1. Besides, this ecoregion could be characterized by a high amount of different volatile compounds, such as 6-methyl-5-hepten-2-one, 2,6-dimethylphenol, terpinolene and nonanal.

Comparing Figs. 1 and 2, some similarities can be found. Samples from Patagonian Forest ecoregion could be distinguished from other regions by their pollen profile and their volatile compounds. According to these results, it could be inferred that the volatile compounds in honey samples might be linked to the pollen profile of the samples and their geographical origin. These hypotheses are analysed in detail in the following section by using chemometric tools.

3.3. Correlations between geographical origin and volatile compounds

Although the volatile composition has been mainly used for the characterization of the floral source of honey, it could also be used to classify honeys according to their geographical origin (Stanimirova et al., 2010).

Argentinean honeys present a broad and varied pollen spectrum. This country extends over 33° of latitude with a wide variety of habitats that result in a great diversity of honeys (Fagúndez & Caccavari, 2006). However, the volatile composition might not only be defined by the pollen profile of the production region, but also by the climatic conditions, and the anthropogenic activity in the ecoregions (sunlight, moisture, use of fumigants, etc.) could influence honey volatile composition (Kaškonienė & Venskutonis, 2010).

In order to analyse the relationship between volatile compounds and production region, a RDA was performed. This multivariate technique allows capturing the linear relationship between response variables (volatile composition) and the matrix of explanatory variables (in this case, geographical origin) (Brogna et al., 2017). All the production regions analysed (Pampa, Parana Delta and Islands, Espinal and Patagonian Forest) showed an adjusted P-value lower than .05 and a VIF < 20. Fig. 3 displays the RDA biplot; the P value associated with this analysis was .0002. Thus, it could be concluded that the volatile composition of Argentinean honeys was significantly related to their geographical provenance.

According to these results, the compounds ethyl 2-phenylacetate and naphthalene are significantly associated with the production ecoregion of Espinal. Naphthalene is regarded as an exogenous compound and in the past (in some countries) it was used as a moth control agent (Tananaki et al., 2005). Although this compound has been found in most of the honeys from Espinal ecoregion, its relative concentration was very low.

On the other hand, compounds 1,3,8-*p*-menthatriene, cyclopropylidenemethylbenzene, 1,1,6-trimethyl-1,2-dihydronaphthalene, 1,2,4-trimethylbenzene, α-pinene and unknown compound 17 could be associated with honeys from the Pampa ecoregion, while 3-methyloctane, Isopropyl 2-methylbutanoate, 2,6-dimethyl-1,6-octadiene, unknown compound 4, cymene and unknown compound 21 were conspicuous in honeys from Parana Delta and Islands ecoregion. It is interesting to mention that some of these compounds are terpenes. It is well known that terpenes are the major constituents of essential oils. However, there are only partial similarities between terpenes of essential oils and honey from the same plant source. This is because terpenes are transformed by bees in their stomach, from which they are transferred to honey. These transformations are related to the temperature and the conditions of the beehive, probably determined by the climatic conditions of the environment (Jerković & Kuś, 2014). This could explain why, in the present work, the presence of terpenes could be directly related to the geographical origin of honey samples.

Unknown compounds number 7, 10, 2, and 11, terpinolene and 1-(1,4-dimethyl-3-cyclohexen-1-yl)ethanone were distinctive of Patagonian Forest ecoregion. It can be seen that the structure of most of these compounds could not be correctly identified. Patagonian Forest is a new production region and little work has been done in order to characterize its honeys. In the present work, unknown compounds 7, 10 and 11 were only found in this region. This observation suggests that honeys from Patagonian Forest might present some specific volatile compounds. Future work should be done in order to provide further information about these volatile organic compounds.

A detailed inspection of Figs. 1 and 3 shows that, although the pollen profile of Espinal and Pampa ecoregions was very similar (Ji = 0.53), the samples could be correctly classified only by their volatile composition. From this result it could be inferred that the chemical composition of honey does not only depend on the floral origin, but also on several factors determined by the geographical origin of the samples. This explains why the composition of honeys from the same

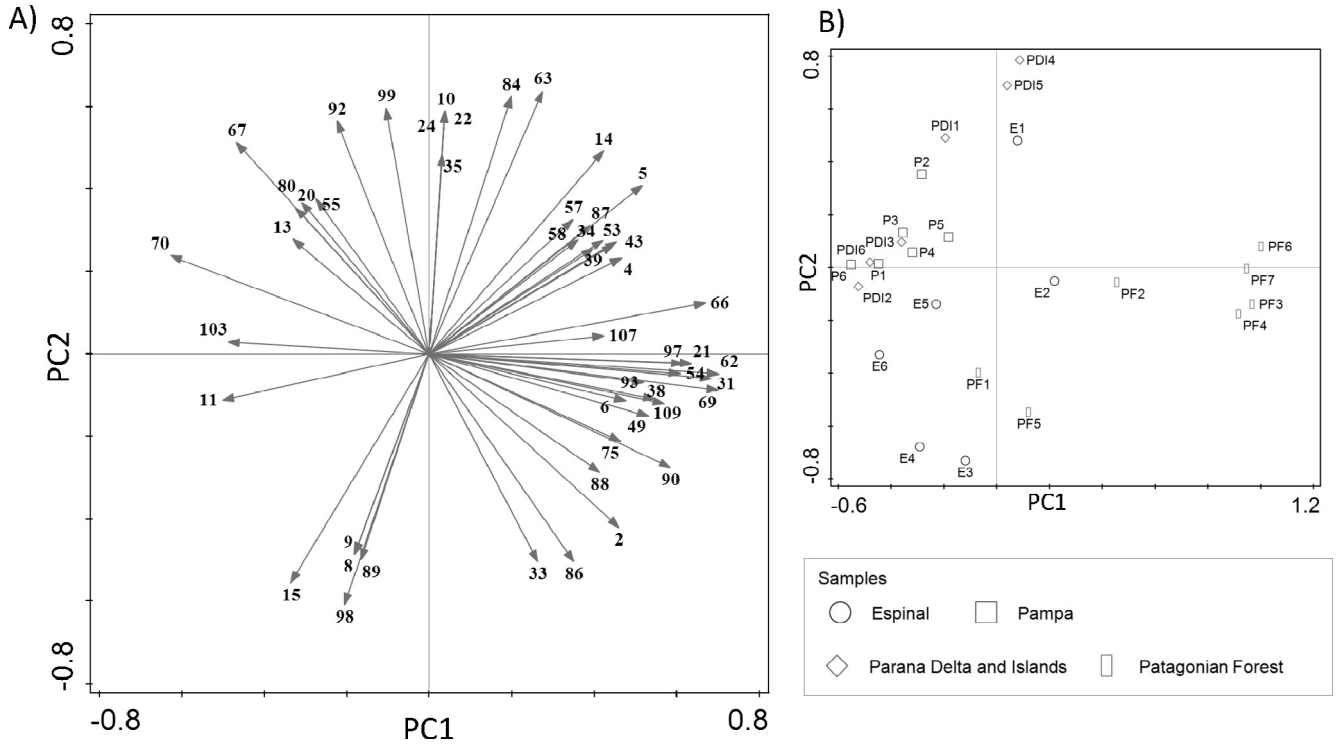

Fig. 2. PCA scatter plot of (A) volatile compounds and (B) honey samples of different ecoregions (Espinal (E), Pampa (P), Parana Delta and Islands (PDI), and Patagonian Forest (PF)). Numbers of the volatile compounds refer to the codes given in Table 1.

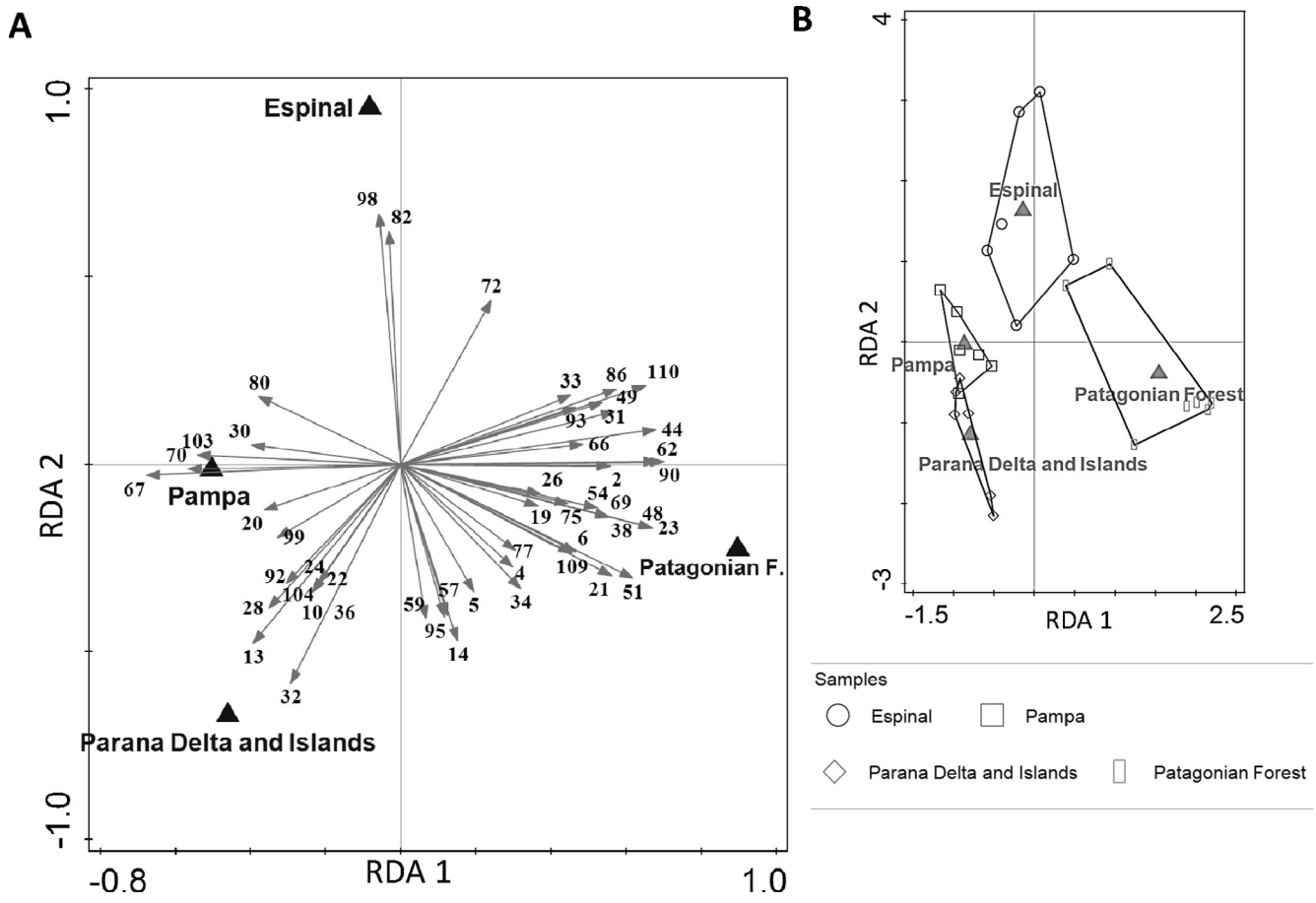

Fig. 3. RDA graphs of honey samples: (A) Biplot of response variables (volatile composition of the samples) and explanatory variables (geographical origin of samples). (B) Classified sample diagram. Numbers of the volatile compounds refer to the codes given in Table 1.

Table 2
Classification matrix for the geographical origin of the honeys according to linear discriminant analysis (LDA) (n = number of samples).

Region	n	Pampa	Patagonian Forest	Parana Delta and Islands	Espinal	Correctly classified samples (%)
Pampa	6	5	0	0	1	83.33
Patagonian Forest	7	0	7	0	0	100
Parana Delta and Islands	6	0	0	6	0	100
Espinal	6	0	0	0	6	100
Total	25	5	7	6	7	96

floral origin may be quite different (Kaškonienė & Venskutonis, 2010). Similar results were found by Karabagias et al. (2017), who analysed citrus honeys from different Mediterranean countries. These authors found significant differences in 15 volatile compounds from different geographical origins but the same major floral source.

In order to find whether honeys from the different ecoregions analysed could be correctly classified according to their volatile composition, a linear discriminant analysis (LDA) was performed. The chosen variables were the ones previously selected by the RDA analysis as the most related to the geographical origin of the honey samples. LDA created a model that included 1,3,8-*p*-menthatriene; cyclopropylidenemethylbenzene; 1,1,6-trimethyl-1,2-dihydronaphthalene; 1,2,4-trimethylbenzene; α-pinene; isopropyl 2-methylbutanoate; cymene; 2,6-dimethyl-1,6-octadiene; 3-methyloctane; 1-(1,4-dimethyl-3-cyclohexen-1-yl)ethanone; terpinolene; ethyl 2-phenylacetate; naphthalene and unknown compounds 17, 4, 21, 7, 10, 2 and 11. Although naphthalene is an exogenous agent, because of its difficulty to be removed, it was also considered in the statistical analysis (Tananaki et al., 2005).

The obtained LDA presented an excellent predictive capacity according to the misclassification table (Table 2). The first two dimensions (CV1 and CV2) accounted for 99.93% of the data variance, and the Wilks' Lambda value for the proposed model indicates a good discriminating power ($P < .05$). The only sample that could not be correctly classified was P1, which, as explained before, showed some pollen families characteristic of Espinal ecoregion. Considering the volatile composition and the pollen profile of this sample, it could be concluded that P1 could be better classified in the Espinal ecoregion rather than in Pampa ecoregion.

3.4. Correlations between floral origin and volatile compounds

According to the forward selection RDA three pollen types were significantly correlated with the volatile profile of honey samples: *Eucalyptus* sp. (adjusted P-val = .00175), *Aristotelia chilensis* (adjusted P-val = .005) and T. *Baccharis* (adjusted P-val = .0175) (Fig. 4). The presence of pollen from *Aristotelia chilensis* could be associated with compounds such as (−)-4-terpineol, 1-methoxy-4-methylbenzene, undecanal and 1-(1,4-dimethyl-3-cyclohexen-1-yl)ethanone. T. *Baccharis* could be well characterized by the presence of compounds such as ethyl 2-methylbutanoate, ethyl 2-phenylacetate, ethyl octanoate and unknown compound 3. The *Baccharis* pollen type is broad: it comprises species of the *Baccharis* and *Eupatorium* (shrub genera) and *Solidago chilensis* (herbaceous specie). The genus *Baccharis* L. is the richest in species within the Astereae tribe, their number being estimated between 400 and 500. Its geographical distribution is exclusively American: it extends from the south of the United States of America to the southern tip of Argentina and Chile. The genus *Baccharis* is represented in Argentina by 96 species (Giuliano, 2001). On the other hand, the genus *Eupatorium* is distributed in temperate and warm regions of the globe, mainly America, and in Argentina is represented by 82 species, which, given their propensity for inhabiting temperate or warm

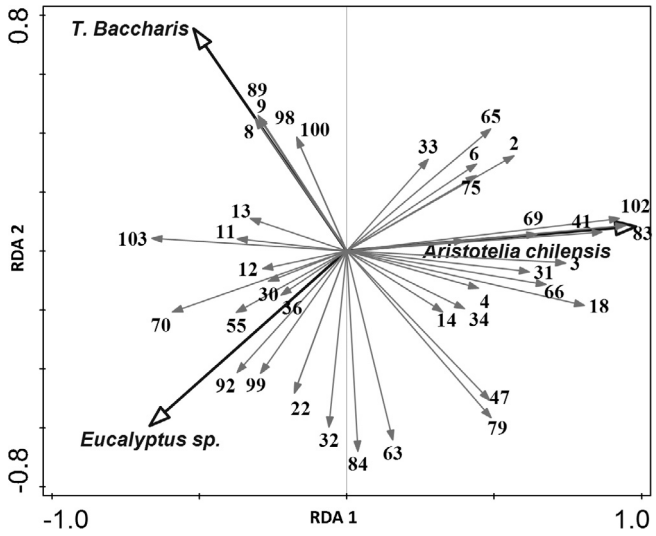

Fig. 4. RDA biplot of response variables (volatile composition of the samples) and the selected explanatory variables (floral origin). Numbers of the volatile compounds refer to the codes given in Table 1.

regions, are likely to be associated with the Pampa, Espinal and Parana Delta and Islands ecoregions. Honeys from *Eucalyptus* sp. have been extensively studied, and several compounds have been proposed as floral markers (Kaškonienė & Venskutonis, 2010). According to the present results, α-ionene could be a marker of *Eucalyptus* sp. pollen. In line with this finding, previous work indicated that in *Eucalyptus* monofloral honeys from Spain and Greece oxo-α-ionone was the most abundant volatile compound and could be considered a good marker of floral origin (Alissandrakis, Tarantilis, Pappas, Harizanis, & Polissiou, 2011; Vázquez, Díaz-Maroto, Guchu, & Pérez-Coello, 2006). Norisoprenoids in honey appear to be originated from the floral source. However, biosynthetic interconversion among the norisoprenoids is also possible depending on the conditions of the bee hive. It has been indicated that intramolecular acid-catalyzed conjugate addition may take place in the acidic medium of the hive (Jerković & Kuś, 2014). These interconversions could lead to slight differences in compounds that may be considered as markers of *Eucalyptus* sp. pollen. Evidence in the present work suggests that derivatives of α-ionone, such as α-ionene and oxo-α-ionone, could be considered markers of the presence of *Eucalyptus* pollen in honeys of different geographical origin (the structures of these compounds are displayed in Fig. S1, Supplementary material).

4. Conclusions

A complete analysis of the volatile compounds and pollen profile of Argentinean honeys from different regions was performed. Besides, a procedure to evaluate possible correlations of the floral/geographical origins with the volatile compounds in honey was fully described. This procedure could help to guarantee the correct classification of honeys and the selection of volatile markers from floral origin.

Significant correlations were found between the geographical origin of honey samples and their volatile composition. It could be concluded that volatile compounds 3,8-*p*-menthatriene; cyclopropylidenemethylbenzene; 1,1,6-trimethyl-1,2-dihydronaphthalene; 1,2,4-trimethylbenzene; α-pinene; isopropyl 2-methylbutanoate; cymene; 2,6-dimethyl-1,6-octadiene; 3-methyloctane; 1-(1,4-dimethyl-3-cyclohexen-1-yl)ethanone; terpinolene; ethyl 2-phenylacetate; naphthalene and unknown compounds 17, 4, 21, 7, 10, 2 and 11 could be used as geographical markers of Argentinean honeys. The present results indicate that an excellent classification of honey samples can be achieved considering only the volatile composition of honey samples of different floral origins.

A total of 23 volatile compounds could not be identified in the present work; future studies should be done in order to provide further information about the structures of these volatile compounds and their origin.

Moreover, the statistical analysis did show significant correlations between some particular floral origins and the volatile compounds in honeys from different ecoregions (*Eucalyptus* sp., *Aristotelia chilensis* and T. *Baccharis*).

These results indicate that honey volatile composition in combination with chemometric tools can successfully be used to find markers of geographical and floral origin. Moreover, the present evidence suggests that there is no need to preselect monofloral honeys in order to find correct markers of floral origin. Nonetheless, a proper reduction of variables is mandatory.

Acknowledgments

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.11.010.

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