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Volatile and carbohydrate composition of rare unifloral honeys from Spain

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Abstract

Volatile composition and carbohydrate content of Spanish honey samples from uncommon botanical origins have been studied by gas chromatography coupled to mass spectrometry. About 100 volatile compounds were identified; some of them appeared to be characteristic of particular honey types, such as methyl salycilate in willow (*Salix spp.*), 2,6,6-trimethyl-2,4-cycloheptadien-1-one (eucarvone) in almond tree (*Prunus dulcis*) and isophorone in strawberry-tree (*Arbutus unedo*). Concentration ranges for major carbohydrates were similar to those previously reported in other honeys with different botanical origins, although concentrations of maltulose in avocado honeys (*Persea americana*) and of melezitose in *Quercus ilex* honeys were higher. Some carbohydrate alcohols could also be considered as markers of honey botanical origin, such as quercitol for *Q. ilex* and perseitol for avocado.

Keywords: Honey; Carbohydrates; Volatile compounds; Polyalcohols; GC–MS

1. Introduction

European Community legislation on honey packaging recommends the use of labels indicating floral and geographical origin, as well as specific quality criteria (Council Directive 2001/110/EC, 2002). The determination of botanical source of honey responds to consumer demands and guarantees the quality of the products, avoiding frauds. Thus an extensive characterization of honey samples becomes a necessary task.

Taste and flavour are two of the most significant attributes of honey. Sweet taste is mainly due to sugars which account for around 80% of honey, but acid and bitter notes are also present. Aroma is produced by complex mixtures of volatile compounds, which vary, depending on nectar origin, processing and storage conditions. Both attributes contribute to the quality and may also help in the authentication of honeys (Arvanitoyannis, Chalhoub, Gotsiou, Lydakis-Simantiris, & Kefalas, 2005).

Honeys produced in Spain in high quantities, such as eucalyptus, heather, lavender, thymus, citrus, rosemary and honeydew have been extensively studied (Pérez-Arquillué, Conchello, Ariño, Juan, & Herrera, 1995; Sanz, González, de Lorenzo, Sanz, & Martínez-Castro, 2004; Serra-Bonvehí, Gómez Pajuelo, & Gonell Galindo, 1987; Soria, González, de Lorenzo, Martínez-Castro, & Sanz, 2005). On the contrary, there are very few data about composition of less common Spanish nectar and honeydew honeys; a special case is that of honeys from the Canary Islands, where vegetable endemisms are very abundant: hence several monofloral honeys are produced only in this region (Serra-Bonvehi, Bentabol-Manzanares, & Santos-Vilar, 2004).

Recently, some chromatographic methods for analyzing both volatile compounds (De la Fuente, Martínez-Castro, & Sanz, 2005; Soria, Martínez-Castro, & Sanz, 2003) and carbohydrates (De la Fuente, Sanz, Martínez-Castro, & Sanz, 2006; Sanz, Sanz, & Martínez-Castro, 2004a, 2004b) in honeys by GC–MS have been improved and new compounds have been identified.

In this work, as part of a project on characterization of Spanish honeys, a detailed study of volatiles, polyalcohols

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and carbohydrates of some rare honeys commercially available in Spain has been carried out. Most of the data about volatile and carbohydrate composition of these honey types are presented for first time.

2. Materials and methods

2.1. Honey samples

Honey samples were from *Persea americana* (avocado, code; AV), *Arbutus unedo* (strawberry-tree; code: STRW), *Salix* spp. (willow; code: WIL), *Eriobotrya japonica* (loquat; code: LOQ), *Prunus dulcis* (almond tree; code: ALM), *Abies* spp. (fir; code: ABIES), *Quercus ilex* (evergreen oak; code: OAK), *Anthyllis cytisoides* (code: CYT), *Satureja montana* (code: SAV), *Spartocytisus supranubius* (Teide broom; code: TBR), *Agave* spp. (agave; code: AG), and *Echium wildpretii* (tajinaste; code: TAJ). These last three are characteristic products from the Canary Islands. Three multifloral honeys (code: MF) elaborated in the Canary Islands were also included in this study, since they were probably different from other multifloral honeys from Spain.

2.2. Analysis of volatile compounds

Analysis of volatile compounds was carried out by SPME, using a Carboxen/PDMS fibre from Supelco (Bellefonte, PA), as previously described by Soria et al. (2003). GC–MS analyses were carried out on an Agilent 6890 (Palo Alto, CA) gas chromatograph coupled to an Agilent 5973 quadrupole mass spectrometer detector (Palo Alto, CA) (De la Fuente et al., 2005).

Retention and spectral data were compared with those of standard compounds or with published retention indices and Wiley mass spectra library (Wiley, 1989). Semiquantitative data were calculated from TIC peak areas as percentage values, assuming a similar response for all volatile compounds.

2.3. Carbohydrate analysis

GC carbohydrate analysis was carried out using a two-step derivatization procedure (oximation and trimethylsilylation) which was applied to both standards and samples (Brobst & Lott, 1966; Li & Schumann, 1981). GC analysis was carried out according to Sanz, González, de Lorenzo, Sanz, and Martínez-Castro (2005) using a methyl silicone column.

GC–MS analyses were carried out using the same capillary column installed in a HP-6890 chromatograph with a MSD 5973 quadrupole mass detector (both from Hewlett–Packard) working in EI mode at 70 eV. Helium was used as carrier gas, and injections were made in split mode, with a split flow of 40 ml min^{-1}. Acquisition was done using HPChem Station software (Hewlett–Packard, Palo Alto, CA, USA).

Identification of peaks was carried out by comparing their retention times with those of standard compounds; mass spectral data (Sanz, Sanz, & Martínez-Castro, 2002) were used to confirm peak identities. The derivatization procedure previously described gives rise to a single chromatographic peak for non-reducing sugars, corresponding to their trimethylsilyl ethers, whereas two peaks are detected for reducing sugars, corresponding to their oxime isomers *Z* and *E*.

Quantitative values were calculated from FID peak areas using the internal standard method. Standard solutions containing different proportions of each carbohydrate were prepared to calculate the response factor (RF) relative to phenyl-β-D-glucoside (internal standard) over the expected range. Concentration of cyclitols was estimated by assuming a response factor = 1 due to the lack of some standards. It is well known that monosaccharides (glucose and fructose) and trisaccharides usually appear as clearly separated peaks and these were directly quantified, whereas disaccharide fraction is never completely resolved. Thus, for quantitative analysis of disaccharides, it was necessary to use an iterative method recently developed in our laboratory (De la Fuente et al., 2006).

3. Results and discussion

3.1. Volatile composition

About 100 volatile compounds were identified in honeys. Some of them, present in medium and even high relative amounts, were not quantified for different reasons. Phthalates, aliphatic hydrocarbons (C15–C17) and several linear alkyl benzenes (LABs), appearing in different samples, were considered as pollutants (Bentivenga, D'Auria, Fedeli, Mauriello, & Raccioppi, 2004; Eganhouse, 1986). Although small quantities of trimethyl phenols have been reported in some honey samples (Castro-Vazquez, Perez-Coello, & Cabezudo, 2003), their origin is unclear and they were not taken into account. Furfural depends on heat treatment and ethanol may be related to the development of yeasts (Beckh, Wessel, & Luellman, 2005; Papoff, Campus, Floris, & Farris, 1995). The origin of 2-ethyl hexanoic acid is also uncertain: although this compound has been identified among volatile components of wines and beers (Vinh, Schwartz, & Moll, 1981) and as constituent of *Cephalophora aromatica* from Iran (Sefidkon & Omidbaigi, 2004), it has also been reported as an industrial product used as stabilizer, plasticizer, refrigerating lubricant, rubber activator, dyestuff, antiseptic and a metabolite from phthalates (Lampen, Zimnik, & Nau, 2003). Table 1 summarizes the most important volatile components quantified in every type of honey.

P. americana honeys (AV) had high relative amounts of dimethyl sulfide (38.5–74.4%) This compound has also been detected in other honeys (De la Fuente et al., 2005) at lower concentration. It was always accompanied by small proportions of dimethyl sulfoxide, which could be

Table 1
Main relative volatile composition (%) characteristics of each honey origin

Samples	Compounds	% (range)
AV ($n = 5$)	Dimethyl sulfide	38.5–74.4
	1-(2-Furanyl)ethanone	0.0–27.9
	3-Methyl 1-butanol	4.2–7.7
	Benzaldehyde	1.5–12.0
	2,3-Butanedione	0.0–14.8
MF ($n = 3$)	Dimethyl sulfide	0.9–65.7
	Linalool oxide (*cis*)	0.4–35.5
	2,3-Butanedione	4.4–17.5
	3-Methyl-1-butanol	7.0–8.3
	Phenylacetaldehyde	0.4–13.0
TAJ ($n = 3$)	Linalool oxide (*cis*)	2.2–48.2
	2,3-Butanedione	4.1–21.0
	Phenylacetaldehyde	4.9–13.9
	2-Ethyl-1-hexanol	0.0–16.0
	3-Methylbutanal	0.0–16.3
TBR ($n = 2$)	2,3-Butanedione	24.6–31.7
	Dimethyl sulfide	0.0–25.2
	Benzaldehyde	8.3–9.9
	3-Methyl-1-butanol	4.2–13.2
	2-Phenylethanol	2.3–7.7
OAK ($n = 2$)	Dimethyl sulfide	7.4–33.7
	3-Methyl-1-butanol	6.3–12.3
	3-Methyl-3-buten-1-ol	7.7–10.7
	Linalool oxide (*cis*)	3.4–8.6
	Isophorone	0.6–10.9
STRW ($n = 2$)	Isophorone	76.2–81.1
	2-Hydroxy-3,5,5-trimethyl-2-ciclohexenone	6.8–7.0
	4-Oxoisophorone	2.5–4.7
	2-Furanmethanol	0.0–2.3
	Dimethyl sulfide	0.0–2.2
LOQ ($n = 3$)	3-Butanenitrile (tentative)	0.0–27.2
	Dimethylsulfide	3.2–25.9
	Benzaldehyde	0.9–28.0
	Dimethyl disulfide	0.0–17.1
	Eucarvone	0.0–16.6
ABIES ($n = 1$)	Butanoic acid	16.5
	Methyl butyrate	10.1
	α-Pinene	8.2
	α-Phellandrene	3.4
	1,8-Cineole	3.1
SAV ($n = 1$)	2-Phenylethanol	24.4
	Dimethyl sulfide	22.8
	3-Methyl-1-butanol	9.6
	Benzaldehyde	6.5
	Nonanal	4.3
CYT ($n = 1$)	Phenylacetaldehyde	80.7
	Benzaldehyde	6.9
	1-(2-furanyl)ethanone	3.3
	2-Phenylethanol	2.8
	Linalool oxide (*cis*)	1.9
ALM ($n = 1$)	Eucarvone	63.7
	RI = 1981; *m/z* (123, 138, 151, 166)[a]	9.2
	4-Oxoisophorone	8.3
	Isophorone	5.1
	Benzaldehyde	4.5
AG ($n = 1$)	2-Phenylethanol	28.6
	Dimethyl disulfide	19.4
	3-Methyl butanoic acid	16.3
	3-Methyl 1-butanol	10.2
WIL ($n = 1$)	Phenylacetaldehyde	25.3
	Benzaldehyde	22.3
	Methyl salycilate	11.1
	2,6,6-Trimethyl 2,4-cycloheptadien-1-one	7.4
	Benzyl alcohol	6.1

Codes are described in Section 2. n = number of samples for a honey type.
[a] Unidentified compound.

formed from dimethyl sulfide by the action of hydrogen peroxide generated by glucose oxidase (Castro-Vazquez et al., 2003). Dimethyl sulfide content did not correlate with those of dimethyl disulfide and dimethyl trisulfide.

E. wildpretii (TAJ) showed a variable composition, but high values of linalool oxides, 2,3-butanedione and phenyl acetaldehyde (average values 28.1%, 14.2% and 8.5%, respectively) were found. These compounds have pleasant flavours and could contribute to the aroma.

Canarian multifloral honeys were different from other Spanish multifloral samples previously studied (unpublished data). Volatile composition of two of the Canarian multifloral samples was similar to *P. americana* honeys from this zone. As *P. americana* is extensively cultivated in the Canary Islands, its contribution to multifloral honeys of these Islands was expected. The third multifloral sample presented a high amount of linalool oxides, which could be explained by some contribution of *E. wildpretii* nectar.

2,3-Butanedione was a major component (24.6–31.7%) in the two analyzed samples of *S. supranubius* honey (TBR), followed by benzaldehyde, 3-methyl-1-butanol and 2-phenylethanol. Dimethyl sulfide only appeared in one sample in a high concentration (25.2%). Presence of α-pinene (traces) and β-pinene (2.2%) in other samples could be attributed to the presence of some honeydew (see below).

The chromatographic profiles of *Q. ilex* honeys (OAK) showed an intense peak corresponding to acetic acid. It is reported that this oak emits acetic acid to the atmosphere (Kesselmeier, Bode, Gerlach, & Jork, 1998). High concentrations of dimethyl sulfide, 3-methyl-1-butanol and 3-methyl-3-buten-1-ol were also found.

Isophorone was the most abundant compound in *A. unedo* samples (STRW) (76.2–81.1%); 2-hydroxy-3,5,5-trimethyl-2-cyclohexenone was also detected in relatively high concentrations (6.8–7%) and 4-oxoisophorone appeared in lower proportions (2.5–4.7%). Bianchi, Careri, and Musci (2005) suggested the use of some norisoprenoid compounds (α- and β-isophorone and 4-oxoisophorone) as specific floral origin markers of 10 Sardinian strawberry-tree honeys. Nor-isoprenoid compounds have been considered as floral markers for *Ericaceae* (Guyot, Scheirman, & Collin, 1999).

Nevertheless, low proportions of isophorone derivatives have been detected in honeys of different floral origins (De la Fuente et al., 2005), and more composition data would be necessary in order to establish ranges for isophorone level in strawberry-tree honeys.

Several short-chain nitriles appeared in two of the three *E. japonica* honey samples (LOQ). High proportions of a compound with formula C_4H_5N (probably 3-butenenitrile or methylpropenenitrile (18.9% and 27.2%)) could be noted. Small amounts of these types of compounds have previously been found in samples of other floral origins (De la Fuente et al., 2005; Soria et al., 2005).

Only one sample each of honeys from *Abies*, *S. montana*, *A. cytisoides, P. dulcis*, *Agave* and *Salix* could be collected. Although the results obtained for these samples are only indicative, some characteristic aspects may be pointed out.

Several terpene hydrocarbons were present in *Abies* honey (ABIES), α-pinene (8.2%), α-phellandrene (3.4%), α-terpinene (2.3%), limonene (1.5%), 1,8-cineole (3.1%) and sabinene (1.6%) being the most abundant. These compounds have been reported in essential oils of conifers (Santos et al., 2006).

2-Phenylethanol was the main compound of *S. montana* (SAV) honey (24.4%), along with dimethyl sulfide (22.8%). Other noticeable components were aldehydes (benzaldehyde, phenyl acetaldehyde, nonanal and decanal), branched alcohols, linalool oxides and acetic acid.

Volatile composition of *A. cytisoides* (CYT) honey was characterized by the extremely high amount of phenylacetaldehyde (80.7%); the sample also contained benzaldehyde (6.9%), nonanal, lilac aldehyde, linalool oxides and 2-phenylethanol.

The most striking feature of *P. dulcis* honey (ALM) was the high concentration (63.7%) of eucarvone (2,6,6-trimethyl-2,4-cycloheptadien-1-one). This compound has been identified in several plants of the *Teucrium* genus (Perez-Alonso, Velasco-Negueruela, & Lopez-Saez, 1993) and in *Crocus sativus* (Maurizio, 2004); it has also been reported (Radovic et al., 2001) in eight heather and three eucalyptus honeys. We found noticeable amounts of this compound (7.4% and 16.6%) in two of the three *E. japonica* honeys. Besides, a compound with retention index (RI) 1979 and mass spectrum compatible with 7-methoxy-1,5,5-trimethylcyclohepta-1,3-diene was also present in almond honey (8.4%); to the best of our knowledge it is the first time that this compound is reported in honeys. Since the proposed structure is very similar to that of eucarvone, the origin of both compounds could be related. Both compounds were found in flowers from *Prunus* sp., but they were not detected in *P. dulcis* flowers.

The *Agave* honey (AG) sample presented the highest proportion of dimethyl disulfide (19.4%) of all analysed honeys; in contrast, other sulfur compounds were very scarce or absent. It also had high proportions of 2-pheny-

Table 2
Carbohydrates and polyalcohols found in the examined honeys

Trivial name	IUPAC name
Sucrose	β-D-Fructofuranosyl-α-D-glucopyranoside
α,α-Trehalose	α-D-Glucopyranosyl-α-D-glucopyranoside
α,β-Trehalose	α-D-Glucopyranosyl-β-D-glucopyranoside
Trehalulose	α-D-Glucopyranosyl-(1→1)-D-fructose
Sophorose	β-D-Glucopyranosyl-(1→2)-D-glucose
Laminaribiose	β-D-Glucopyranosyl-(1→3)-D-glucose
Maltose	α-D-Glucopyranosyl-(1→4)-D-glucose
Maltulose	α-D-Glucopyranosyl-(1→4)-D-fructose
Nigerose	α-D-Glucopyranosyl-(1→3)-D-glucose
Turanose	α-D-Glucopyranosyl-(1→3)-D-fructose
Kojibiose	α-D-Glucopyranosyl-(1→2)-D-glucose
Palatinose	α-D-Glucopyranosyl-(1→6)-D-fructose
Cellobiose	β-D-Glucopyranosyl-(1→4)-D-glucose
Gentiobiose	β-D-Glucopyranosyl-(1→6)-D-glucose
Melibiose	α-D-Galactopyranosyl-(1→6)-D-glucose
Isomaltose	α-D-Glucopyranosyl-(1→6)-D-glucose
1-Kestose	α-D-Glucopyranosyl-(1→2)-β-D-fructofuranosyl-β-D-fructofuranoside
Theanderose	α-D-Glucopyranosyl-(1→6)-α-D-glucopyranosyl-β-D-fructofuranoside
Raffinose	α-D-Galactopyranosyl-(1→6)-α-D-glucopyranosyl-β-D-fructofuranoside
Erlose	α-D-Glucopyranosyl-(1→4)-α-D-glucopyranosyl-β-D-fructofuranoside
Melezitose	α-D-Glucopyranosyl-(1→3)-β-fructofuranosyl-α-glucopyranoside
Panose	α-D-Glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→4)-D-glucopyranose
Maltotriose	α-D-Glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-D-glucopyranose
Quercitol	1,3,4/2,5-Cyclohexanepentol
Methyl-*muco*-inositol	1-*O*-Methyl-*muco*-inositol
Pinitol	3-*O*-Methyl-*chiro*-inositol
Muco-inositol	1,2,4,5/3,6-Cyclohexanehexol
Chiro-inositol	1,2,4/3,5,6-Cyclohexanehexol
Myo-inositol	1,2,3,5/4,6-Cyclohexanehexol
Perseitol	D-G*lycero*-D-*galacto*-heptitol

Table 3
Mono-, di- and trisaccharide contents (mg/g honey) in studied honey samples

Code	AV ($n = 5$)	MF ($n = 3$)	TAJ ($n = 3$)	TBR ($n = 2$)	OAK ($n = 2$)	STRW ($n = 2$)	LOQ ($n = 3$)	ABIES ($n = 1$)	SAV ($n = 1$)	CYT ($n = 1$)	ALM ($n = 1$)	AG ($n = 1$)	WIL ($n = 1$)
Fructose	307–413	361–411	344–405	411–426	371–394	285–378	248–380	331	397	329	339	438	398
Glucose	184–264	242–296	275–324	243–268	249–277	237–266	313–330	242	324	268	300	344	295
Sucrose	0.5–3.9	1.1–2.6	3.2–17.3	0.4–3.6	4.2–11.5	0.0–1.8	0.0–1.0	1.3	1.6	5.2	0.0	1.2	1.7
α,α-Trehalose	0.0–0.7	0.1–0.4	0.0–0.2	0.5–0.3	0.3–1.0	0.0–0.3	0.0–0.5	0.6	0.1	0.0	0.0	0.4	0.0
α,β-Trehalose	4.4–7.3	4.0–5.6	3.0–5.5	3.4–5.3	3.7–5.0	3.8–6.2	3.5–4.2	7.6	3.2	4.7	4.9	5.3	5.8
Cellobiose	0.6–0.9	0.6–0.9	0.8–1.1	0.4–0.5	1.4–1.7	1.0–3.0	1.7–2.2	1.0	1.7	1.7	2.7	1.4	1.6
Laminaribiose	1.0–2.9	1.3–1.9	1.3–2.7	1.2–2.0	1.1–1.2	2.1–4.1	2.8–7.1	2.7	1.7	4.0	4.9	2.9	3.0
Maltulose	29.0–43.4	16.2–41.0	12.3–22.8	29.4–34.2	15.1–22.6	18.5–24.9	15.9–32.3	34.8	5.8	14.4	12.8	14.0	21.0
Nigerose	13.2–18.5	10.9–15.9	9.4–13.4	10.0–13.6	10.1–13.0	11.8–17.1	10.8–16.3	20.2	8.5	12.6	13.5	14.4	16.1
Turanose	23.8–37.8	22.2–30.4	17.6–32.8	21.4–30.9	19.1–22.5	22.4–24.4	16.4–27.1	45.6	8.9	20.0	13.3	23.4	27.1
Maltose	12.8–22.5	11.6–13.4	10.1–27.6	10.5–12.7	20.9–24.1	11.7–12.6	9.7–14.9	13.4	28.0	21.2	22.3	27.1	16.1
Kojibiose	12.4–28.4	15.8–19.4	13.0–22.1	10.3–21.6	17.3–22.2	16.1–22.2	13.5–18.8	29.5	13.7	18.9	18.1	2.4	21.9
Trehalulose	15.9–37.9	8.1–26.5	6.0–13.1	16.8–20.5	6.6–9.8	9.7–16.7	9.7–18.2	21.5	2.7	8.1	7.1	7.1	11.7
Palatinose	3.9–8.9	2.5–7.0	1.6–3.4	4.1–4.5	1.7–3.1	2.5	2.8–5.6	5.8	0.9	2.0	1.6	1.7	3.1
Gentibiose	0.1–0.7	0.1–0.7	0.1	0.1–0.3	0.3–0.6	0.1	0.1	0.0	0.1	0.1	0.1	1.5	0.1
Isomaltose	23.5–39.6	11.2–40.6	7.8–15.5	22.7–25.2	8.7–13.4	11.4–25.5	10.4–21.5	31.1	4.3	11.1	10.0	10.1	15.5
Melibiose	0.0–0.4	0.0–0.5	0.0	0.1–0.2	0.2	0.0	0.0–1.1	0.0	0.0	0.0	0.0	0.2	0.0
Unknown	0.0–0.9	0.3–2.1	0.0–4.1	0.0–2.0	4.7–5.5	0.0–0.4	0.0–1.9	0.7	0.0	1.7	0.1	0.0	0.0
Raffinose	0.1–0.4	0.0	0.0	0.2–0.5	0.6–1.5	0.0–0.2	0.0	0.0	0.2	0.0	0.1	0.4	0.0
Kestose	0.7–1.6	0.8–1.8	1.3–2.3	0.1–0.8	3.0–4.2	0.0–3.3	0.0–0.9	1.9	0.6	1.3	0.3	2.2	2.3
Erlose	0.7–11.8	5.1–12.4	4.6–17.9	1.5–27.6	6.9–10.0	3.6–7.1	0.0–6.3	6.1	0.7	5.9	0.4	4.5	5.6
Melezitose	1.1–2.2	1.1–2.0	0.6–1.7	0.1–1.6	2.6–3.4	0.0–1.1	0.0–0.9	1.5	0.4	0.6	0.1	2.6	1.4
Theanderose	1.2–1.7	0.7–1.3	0.4–1.5	1.7–2.9	0.4–0.7	0.0–0.5	0.0–0.8	1.4	0.0	0.5	0.0	0.5	1.1
Unknown	0.9–1.4	0.5–1.0	0.0–0.5	0.78–0.81	0.6–1.4	0.0–0.6	0.2–0.5	1.5	0.0	0.0	0.3	0.5	0.0
Maltotriose	1.3–2.1	1.0–1.7	0.8–1.4	1.0–1.7	1.2–1.3	0.0–1.2	1.0–1.8	2.6	0.7	0.0	0.6	1.5	0.0
Unknown	3.1–5.2	1.1–4.2	0.7–1.7	2.2–4.0	0.9–1.6	0.0–1.1	1.2–2.7	4.2	0.0	0.0	0.7	0.9	0.0
Panose	3.2–5.4	1.5–3.7	1.3–3.2	3.0–5.3	1.2–1.9	0.0–1.7	1.8–3.8	3.5	0.3	0.0	0.6	1.9	0.0

Codes are described in Section 2. n = number of samples for a honey type.

lethanol (28.6%), 3-methyl butanoic acid (16.3%) and 3-methyl-1-butanol (10.2%).

Salix honey (WIL) presented methyl salicylate in a high concentration (11.1%). This compound can be used for the treatment of varroa: however, it has not been detected in other analyzed Spanish honeys and it probably comes from *Salix*, a well-known source of salicylic acid and derivatives. Very small amounts of ethyl salicylate and 2-hydroxy-benzaldehyde also appeared in this honey. Other major compounds of *Salix* honeys were phenylacetaldehyde and benzaldehyde (25.3% and 22.3%, respectively), which have been reported in other honeys (De la Fuente et al., 2005).

3.2. Carbohydrate composition

Fifteen disaccharides and seven trisaccharides were detected in the studied honeys (Table 2): in addition, three unidentified compounds which were supposed to be trisaccharides on the basis of their retention times and mass spectra were also determined (Fig. 1). Sugar concentrations are shown in Table 3.

Sugar composition has been used to discriminate honey samples by botanical origin (Cotte, Casabianca, Chardon, Lheritier, & Grenier-Loustalot, 2004) or geographical provenience (Gómez Bárez et al., 2000). However, several researchers conclude that sugar composition alone is not enough to discriminate among honeys (Bogdanov & Baumann, 1988; Földhazi, 1994). Glucose and fructose contents were in most cases within the limits established by EU legislation (Council Directive 2001/110/EC). Some values slightly lower (*E. japonica* (LOQ), *A. unedo* (STRW) and *P. americana* (AV)) could correspond to mixtures with honeydew honey. *A. unedo* honeys presented the lowest values whereas *Agave* honey (AG) showed the highest ones.

The highest di- and trisaccharide concentrations appeared in *P. americana* (AV) (219 and 26.7 mg/g, respectively) and *Abies* (215 and 22.8 mg/g, respectively) honeys, whereas *P. dulcis* (ALM) and *S. montana* honeys (SAV) presented very low values (111 and 81.0 mg disaccharides/g honey and 3.0 and 2.8 mg trisaccharides/g honey, respectively).

P. americana honeys showed the highest values of maltulose, trehalulose and palatinose among the studied honeys. One multifloral honey from the Canary Islands also presented a similar pattern, with values of maltulose, trehalulose and palatinose relatively high. This may indicate a contribution of *P. americana* honey to Canarian multifloral honey, as was deduced from the volatile composition. Sucrose concentration was low in all studied samples and the maximum levels were within the limits established by the EU legislation (Council Directive 2001/110/EC). All the samples presented α,β-trehalose in higher amounts than α,α-trehalose. The lowest value of maltulose was found in *S. montana* honey which in turn showed the highest maltose content (28.0 mg/g). Turanose was noticeably high

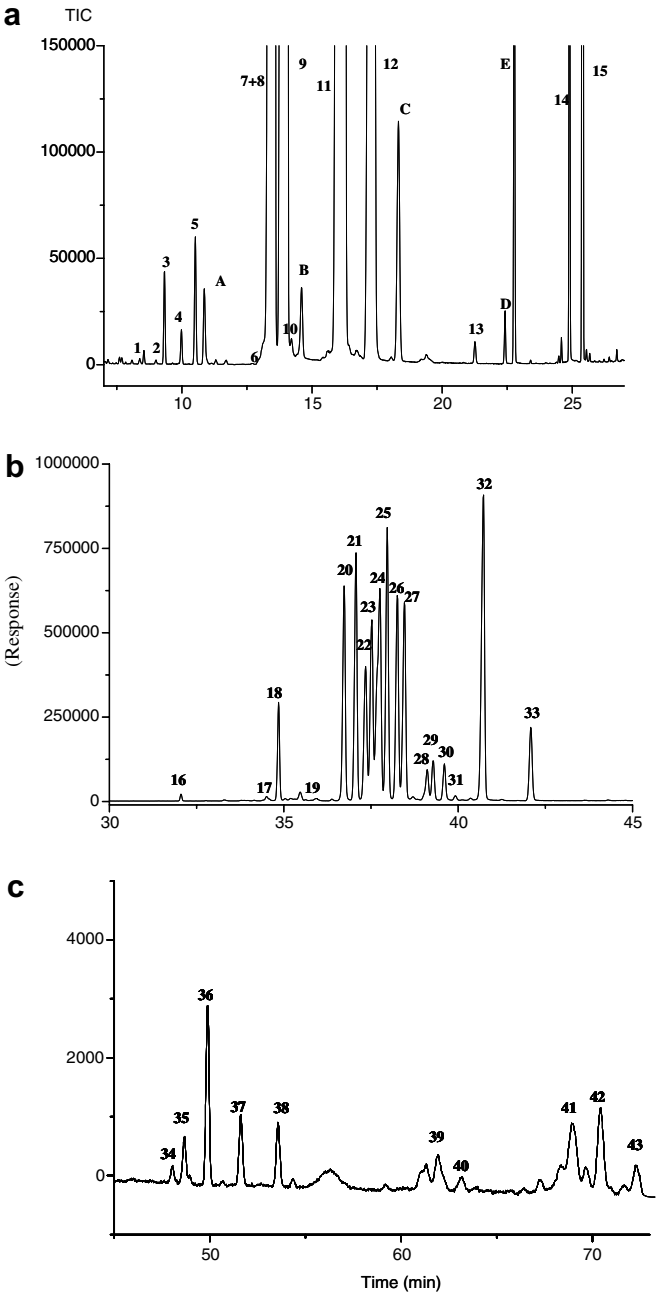

Fig. 1. Gas chromatographic profile of TMS oxime carbohydrates of avocado honey: (a) Monosaccharide and polyalcohol fractions. See text for chromatographic conditions. 1 – Quercitol, 2 – methyl-*muco*-inositol, 3 – pinitol, 4 – quinic acid, 5 – gluconic acid, 6 – *muco*-inositol, 7 – mannitol, 8 and 9 – fructose, 10 – *chiro*-inositol, 11 and 12 – glucose, 13 – *myo*-inositol, 14 – perseitol,15 – phenyl-β-D-glucoside. A, B, C, D and E – unknown. (b) Disaccharide fraction: 16 – sucrose, 17 – α,α- trehalose, 18 – α,β-trehalose, 19 – cellobiose (*E*), 20 – cellobiose (*Z*) + laminaribiose (*E*) + maltulose 1, 21 – maltulose 2; 22 – nigerose (*E*), 23 – turanose 1, 24 – laminaribiose (*Z*) + turanose 2 + maltose (*E*), 25 – kojibiose (*E*); 26 – maltose (*Z*) + trehalulose 1, 27 – nigerose (*Z*) + trehalulose, 28 – palatinose 1 + gentiobiose (*E*), 29 – kojibiose (*Z*), 30 – palatinose 2, 31 – gentiobiose (*Z*), 32 – isomaltose (*E*), 33 – isomaltose (*Z*). (c) Trisaccharide fraction: 34 – raffinose, 35 – 1-kestose, 36 – erlose, 37 – melezitose, 38 – theanderose, 39 – maltotriose (*E*), 40 – maltotriose (*Z*), 41 – unknown, 42 – panose (*E*), 43 – panose (*Z*).

(45.6 mg/g) in *Abies* which also displayed the highest value of kojibiose. *Q. ilex* (OAK) presented high raffinose (1.5 mg/g), kestose (4.2 mg/g) and melezitose (3.4 mg/g) concentrations. This last sugar has been reported to be characteristic of honeydew honeys (Bogdanov & Baumann, 1988). Mass spectra of some trisaccharides are shown in Fig. 2.

3.3. Polyalcohols

Some minor components (polyalcohols and cyclitols; see Table 2) were also determined, along with carbohydrates in the same GC runs (Fig. 1a): the most interesting data are collected in Table 4. Organic acids, amino acids and some artefacts, which appeared as small peaks in the elution zone of polyalcohols and cyclitols, were not determined.

Myo-inositol, previously identified in honeys by Horvath and Molnar-Perl (1998), was found in all the samples in a wide range: from 0.1 mg/g in *A. unedo* honey (STRW) to 2.2 mg/g in *Salix* honey (WIL). Mannitol (Horvath & Molnar-Perl, 1998) was probably also present in all samples, but it could not be quantified when it appeared in concentrations below 0.1 mg/g, due to overlapping with the

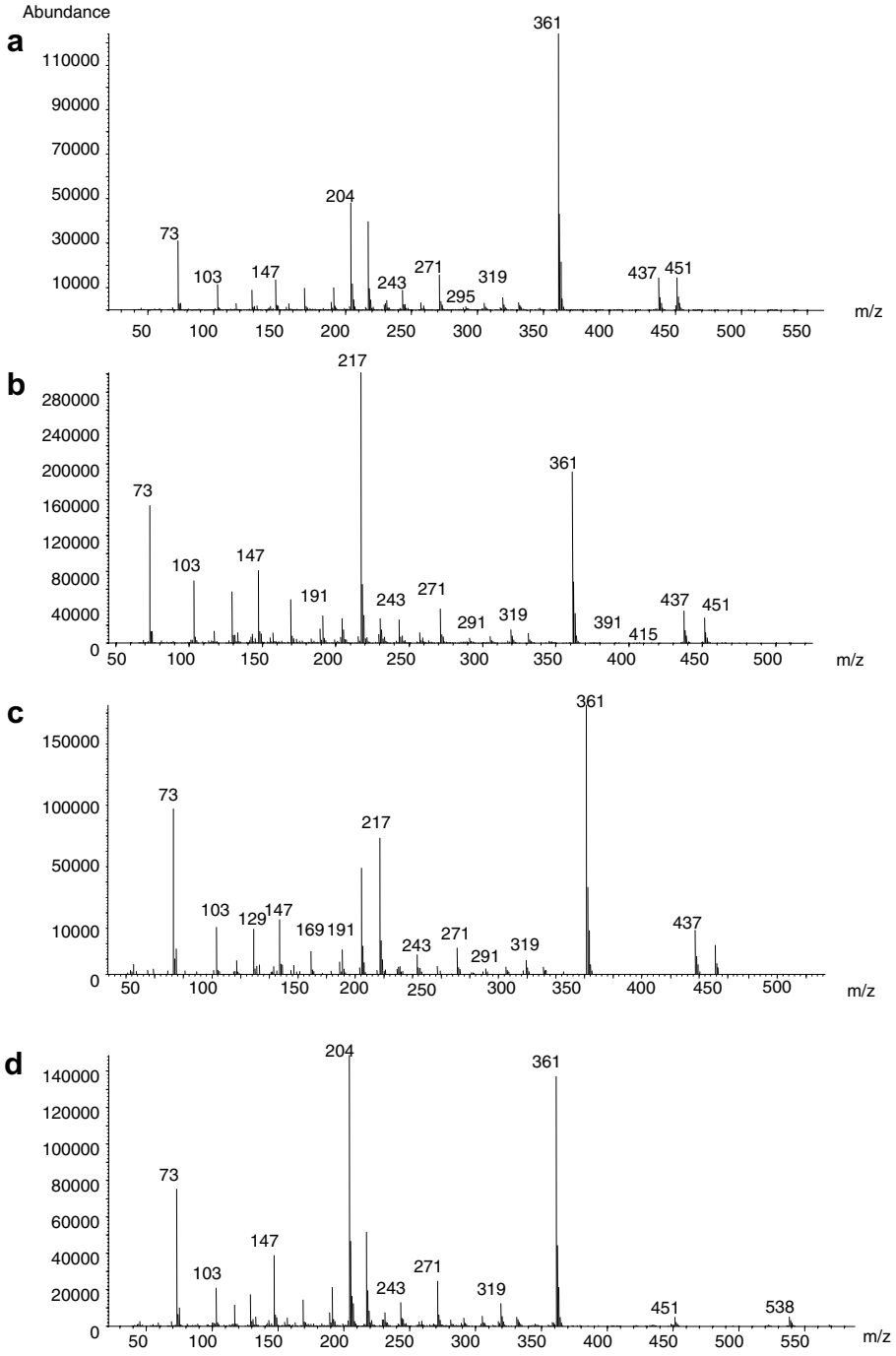

Fig. 2. Mass spectra of TMS-oximes of erlose (a), 1-kestose (b), theanderose (c) and maltotriose (d).

Table 4
Mean contents of polyalcohols (mg/g honey) in the studied honey samples

Codes	AV ($n = 5$)	MF ($n = 3$)	TAJ ($n = 3$)	TBR ($n = 2$)	OAK ($n = 2$)	STRW ($n = 2$)	LOQ ($n = 3$)	ABIES ($n = 1$)	SAV ($n = 1$)	CYT ($n = 1$)	ALM ($n = 1$)	AG ($n = 1$)	WIL ($n = 1$)
Quercitol	0.0	0.1	0.0	0.0	3.6	2.5	0.1	0.0	0.0	0.0	0.5	0.0	0.0
Methyl-*muco*-inositol	0.1	0.8	0.4	0.0	0.6	0.1	0.1	0.1	0.3	0.0	0.1	0.1	0.0
Pinitol	2.1	0.8	0.7	2.9	1.0	0.3	0.2	3.0	0.0	1.3	0.1	0.3	0.0
Muco-inositol	0.0	0.0	0.0	0.0	0.1	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0
Chiro-inositol	0.1	0.0	0.4	0.1	n.q.[a]	2.0	0.3	0.0	0.0	0.8	0.8	0.0	0.7
Mannitol	0.7	0.9	1.3	0.7	2.7	5.8	3.0	0.8	n.q.	0.8	1.2	2.5	n.q.
Myo-inositol	0.6	0.3	0.4	0.2	0.5	0.1	0.5	1.1	0.7	0.9	1.0	0.5	2.2
Perseitol	7.5	4.1	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Codes are described in Section 2. n = number of samples for a honey type.
[a] Non-quantifiable.

first fructose peak. Its level was noticeably high in one *A. unedo* honey (7 mg/g) and in one *E. japonica* (LOQ) sample (10 mg/g).

Pinitol (Sanz et al., 2004a, 2004b) appeared in most samples. This compound is widely distributed in plants and cannot be considered characteristic. 1-*O*-methyl mucoinositol appeared in almost all samples, usually in very small quantities. *Muco*-inositol was detected only in an *E. japonica* sample and in the *Q. ilex* honeys (OAK).

Quercitol (1,3,4/2,5-cyclohexanepentol) was detected in *Q. ilex* honeys in noticeable concentrations (2.3 and 4.9 mg/g honey) supporting previous results which related this compound to the presence of *Quercus* honeydew (Sanz et al., 2005). This compound also appeared in strawberry-tree honeys: however, different parts of a strawberry-tree were analysed by GC–MS but quercitol was not detected. Thus, quercitol could proceed from minute amounts of *Quercus* honeydew in *Arbutus* honey, since both plants appear as neighbours in geographical zones.

Sorbitol has been reported as a characteristic alcohol in *Rosaceae*, and has been proposed as a chemical marker for apple vinegar (Hundley, 1968). Small amounts of this alcohol appeared in *E. japonica* and *P. dulcis* honeys (ALM) (both from Rosaceae) but it was almost impossible to quantify due to a total overlapping with fructose.

Chiro-inositol, which is now reported in honey for the first time, appeared in several samples and its concentration was highest in *A. unedo* honeys. Its chromatographic signal appeared to be partially overlapped by a fructose peak and was difficult to measure in small amounts (<0.1 mg/g).

Perseitol is a well-known specific component of *P. americana* (AV) (Plouvier, 1963); it has previously been suggested as a marker of this honey and it was quantified by both HPLC and infrared spectroscopy, showing values from 0.01% to 1.5% in 109 honey samples from four orchards in Israel (Dag, Afik, Yeselson, Schaffer, & Shafir, 2006). Perseitol is easily detectable by GC using our method since it is eluted in a clean zone of chromatogram between *myo*-inositol and sucrose, near the internal standard (Fig. 1). It was present in all the samples of this botanical origin, where it ranged from 2 to 11 mg/g honey, these values being within the range reported by Dvash et al. (2002). Perseitol also appeared in two multifloral honeys from the Canary Islands, (2 and 6.2 mg/g), confirming the probable presence of noticeable amounts of *P. americana* nectar in these samples, as suspected from their volatile profile; minute amounts of this heptitol were found in other Canarian samples. Polyalcohol pattern of the third Canarian multifloral sample (collected in the mountains) was more similar to those of *S. supranubius* samples (TBR), which were also mountain honeys.

Volatile compounds, carbohydrates and polyalcohols of Spanish honeys from 12 uncommon botanical origins have been studied by GC–MS. Although the results obtained are only indicative, several compounds, some of them reported

for the first time, could be considered as clearly related to the botanical origin of samples. Further studies should be carried out using a higher number of samples.

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