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Occurrence of nitriles in *Taraxacum* labelled honeys

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Abstract

Nitrile compounds, whose presence is not common in honey, have been found in the volatile fraction of a few honey types, their relative amounts being particularly high in honeys commercialized under the *Taraxacum* label. Among them, 2-methylpropanenitrile, 2-methylbutanenitrile, 3-methylbutanenitrile, 2-butenenitrile (*cis*- or *trans*-isomer), 3-butenenitrile and 3-methylpentanenitrile, have been identified for the first time in honeys. Melissopalynological analysis of *Taraxacum* labelled honeys showed a relatively high pollen contribution of other species flowering in spring, such as *Diplotaxis* sp. Nitrogen-containing compounds, such as nitriles, thiocyanates and isothiocyanates have been previously described as products of hydrolysis of glucosinolates present in *Diplotaxis* sp. and other *Brassicaceae*. The nectar contribution of species belonging to the *Brassicaceae* family is proposed as the origin of the high relative amount of nitriles in *Taraxacum* honeys.

Keywords: Honey; Volatiles; Solid-phase microextraction (SPME); GC–MS; Nitriles; *Taraxacum* and *Diplotaxis*

1. Introduction

Honey is appreciated by consumers, not only for its nutritive properties, but also for its special aroma, which is related to its volatile composition. Solid-phase microextraction (SPME) fractionation, followed by GC–MS analysis (Guidotti & Vitali, 1998; Piasenzotto, Gracco, & Conte, 2003; Soria, Martínez-Castro, & Sanz, 2003) has proved to be useful for the identification of the complex mixture of volatiles of different functionality (alkanes, ketones, aldehydes, alcohols, furane derivatives) and molecular weight present in honey. Although GC and MS data unequivocally characterize most of the honey volatile compounds, some components of the volatile fraction in samples from different honey sources have not yet been identified.

Three unidentified compounds with a mass spectrum compatible with a nitrile structure were reported in dandelion (*Taraxacum*) honeys by Bouseta, Collin, and Dufour (1992). Piasenzotto et al. (2003) reported two compounds with the structure of a C5-nitrile and one with the structure of a C6-nitrile, along with phenylacetonitrile, in four dandelion labelled honeys. Nitriles have also been tentatively found at low concentrations in honeys from other sources, such as rape (2-methylpropanenitrile; Radovic et al., 2001), rosemary (3-methylbutanenitrile and phenylacetonitrile; De la Fuente, Martínez-Castro, & Sanz, 2005), loquat (several short-chain nitriles; De la Fuente, Sanz, Martínez-Castro, Sanz, & Ruiz-Matute, 2007) and in Greek cotton honey (neryl and geranyl nitriles; Alissandrakis, Kibaris, Tarantilis, Harizanis, & Polissiou, 2005).

Among the different honeys analysed in our laboratory we have observed that the chromatographic profile of commercial honeys labelled as *Taraxacum* was characterized by several components whose mass spectra were compatible with a nitrile functionality.

The objective of this study is the identification of these nitrile compounds from their chromatographic retention and mass spectral data. A hypothesis for the origin of these

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compounds in *Taraxacum* honeys and in other honeys produced in spring is also proposed.

2. Materials and methods

2.1. Honey samples

This study was carried out using three different Italian commercial honeys labelled as "*Taraxacum*".

2.2. Melissopalynological analysis

Melissopalynological analysis was essentially performed according to Louveaux, Maurizio, and Vorwohl (1978), using the non-acetolytic method. The modifications proposed by Terradillos, Muniategui, Sancho, and Simal-Lozano (1994) were tested and successfully adopted. Microscopic examination was carried out in a Leica DMR light microscope fitted to a digital camera and coupled to an Image Analyser system (Leica Qwin Standard software) for morphometry of pollen grains.

2.3. Standards

3-Methylbutanenitrile, 2-butenenitrile (mixture of *cis*- and *trans*-isomers) and 2-methyl-2-propenenitrile were purchased from Sigma–Aldrich Chemie (Steinheim, Germany); 2-methylbutanenitrile was from ABCR GmbH & Co (Karlsruhe, Germany). Synthesis of 3,3-dimethylbutanenitrile was carried out in a simple two-step reaction from 3,3-dimethylbutanal (Sigma–Aldrich Chemie, Steinheim, Germany) according to the procedure described by Sosnovsky, Krogh, and Umhoefer (1979). A standard of 3-methylpentanenitrile was also synthesized according to Nakamura and Mori (1999). Mixtures of nitrile compounds with other standards were prepared for retention index (RI) calculations.

2.4. Solid-phase microextraction (SPME)

Fractionation of volatiles from honey headspace was carried out by using a manual SPME holder (Supelco, Bellefonte, PA) equipped with a 75 μm Carboxen™-Polydimethylsiloxane fibre (Supelco, Bellefonte, PA). Experimental procedure was as previously described by Soria et al. (2003).

2.5. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analyses were performed on a Hewlett–Packard 6890 (Palo Alto, CA, USA) gas chromatograph coupled to a Hewlett–Packard 5973 quadrupole mass detector.

The SPME fibre was desorbed at 250 °C into a high pressure microseal injector (Merlin, Supelco, Bellefonte, Palo Alto, USA) operating in splitless mode (2 min). Chromatographic separation was carried out on a 50 m × 0.20 mm × 0.20 μm film thickness polyethyleneglycol capillary column (HP-Innowax, Agilent Technologies, USA). The oven was temperature-programmed from 45 °C (2 min) to 190 °C (50 min) at 4 °C min^{-1}. He at ~1 ml min^{-1} was used as carrier gas. The mass detector worked in the EI mode at 70 eV, scanning the 35–450 *m*/*z* range. Interface and source temperature were 280 °C and 230 °C, respectively.

Qualitative analysis was based on the comparison of the obtained mass spectra with those of commercial or synthesized standards, with those of the Wiley mass spectral library (McLafferty & Stauffe, 1989) and with other published data (McLafferty, 1962). It was confirmed, when possible, by coinjection of standards or by using retention indices RI (Piasenzotto et al., 2003; Radovic et al., 2001; Tsuchiya & Sumi, 1977).

Semiquantitative values (percentage of total volatile composition) were directly obtained from total ion current (TIC) peak areas.

3. Results and discussion

3.1. Identification

Fig. 1 shows the TIC profile of the volatile fraction of a *Taraxacum* honey. All the studied honeys were characterized by the presence of several nitrile compounds (peaks A–I); structures were assigned to these compounds by comparison of both RI and mass spectral fragmentation with those of standards and bibliographic data (see Table 1).

For compound A, ions at *m*/*z* 68 $[M-1]^+$, at *m*/*z* 54 $[M-15]^+$ and at *m*/*z* 42 $[M-HCN]^+$ are only compatible with a branched aliphatic nitrile structure, C_4H_7N. Assessment and confirmation of the identification of this compound as 2-methylpropanenitrile was done by comparison with spectra in the Wiley mass spectral library (McLafferty & Stauffe, 1989) and with RI data in the literature (Radovic et al., 2001).

The spectrum of compound B was compatible with a C_5H_9N nitrile structure. From the intensity of the fragments $[M-C_2H_5]^+$ and $[M-C_2H_4]^+$, which seemed to indicate the existence of a methyl substituent in the α-carbon, a 2-methylbutanenitrile structure was tentatively assigned to peak B. Identification was confirmed by coinjection of a commercial standard of 2-methylbutanenitrile.

Compound C was identified as 3-methylbutanenitrile by comparison of its mass spectrum with those in the Wiley mass spectra library and with data reported by McLafferty (1962). Analysis of an authentic sample of *Taraxacum* honey, spiked with a commercial standard of 3-methylbutanenitrile, allowed us to confirm this identification.

A study on the mass spectral fragmentation of compounds D and E, which present a similar spectra (see Table 1), allowed us to tentatively assign a C_4H_5N structure to both compounds. As 2-butenenitrile, 3-butenenitrile and 2-methyl-2-propenenitrile, structures all of them compatible with a C_4H_5N empirical formula, showed an identical mass fragmentation pattern (McLafferty & Stauffe, 1989),

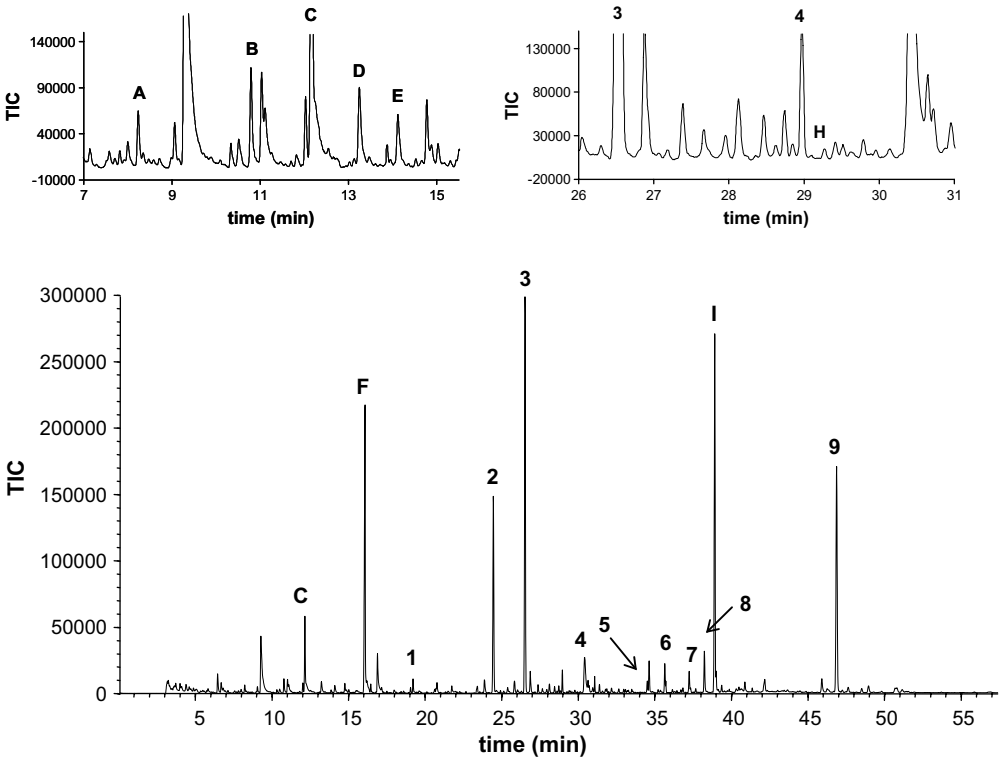

Fig. 1. Chromatographic profile of a *Taraxacum* honey analysed by SPME, followed by GC–MS. Identifications: (A) 2-methylpropanenitrile; (B) 2-methylbutanenitrile; (C) 3-methylbutanenitrile; (D) 2-butenenitrile (isomer not identified); (E) 3-butenenitrile; (F) 3-methylpentanenitrile; (H) benzonitrile; (I) benzeneacetonitrile; (1) 3-methyl-1-pentanol; (2) 2-furancarboxaldehyde; (3) benzaldehyde; (4) benzeneacetaldehyde; (5) methyl ester of 2-hydroxybenzoic acid; (6) β-damascenone; (7) benzenemethanol; (8) benzeneethanol; (9) thymol.

identification of compounds D and E had to rely on chromatographic retention data. Spiking honeys with commercial standards of 2-butenenitrile (mixture of isomers *cis*- and *trans*-) and 2-methyl-2-propenenitrile allowed us to identify compound D as the second-eluting isomer of 2-butenenitrile. Full identification of this compound was not possible as individual standards for each of the isomers of 2-butenenitrile (*cis*- or *trans*-) were not commercially available and there were no data in the literature on the chromatographic retention on polyethyleneglycol for these isomers. Retention time for compound E did not match that of standards of 2-methyl-2-propenenitrile or 2-butenenitrile (first-eluting isomer) and, therefore, compound E was tentatively identified as 3-butenenitrile. Assuming that the elution order of the above mentioned nitriles does not change with the temperature, experimental retention index here calculated for 3-butenenitrile would be in agreement with the retention index data reported by Tsuchiya and Sumi (1977), as 3-butenenitrile would be the C_4H_5N nitrile with higher retention index on polyethyleneglycol.

For compound F, a molecular ion at *m/z* 97 and fragments at *m/z* 96 $[M-H]^+$, *m/z* 82 $[M-CH_3]^+$, *m/z* 68 $[M-C_2H_5]^+$ and *m/z* 57 $[C_4H_9]^+$ are compatible with the structures of both 3-methylpentanenitrile and 3,3-dimethylbutanenitrile. Both compounds were synthesized: the good match between chromatographic retention and mass spectral data from both compound F and the synthesized standard of 3-methylpentanenitrile confirmed the identification.

Compound G with RI = 1253 was present at concentrations lower than 0.20% in *Taraxacum* honeys (see Table 1) and usually overlapped with 3-methyl-3-buten-1-ol. Mass spectral fragmentation of this compound was compatible with a C6 branched nitrile structure. Data from the Wiley mass library (McLafferty & Stauffe, 1989) and the perfect matching between the retention index of compound G and that of a minor impurity, tentatively identified as 4-methylpentanenitrile in the synthesized standard of 3-methylpentanenitrile, allowed a tentative identification of this compound.

Tentative identification of benzonitrile (compound H, RI = 1629) was based on mass spectral data (McLafferty & Stauffe, 1989). The mass spectrum (McLafferty & Stauffe, 1989) of compound I (RI = 1954) and retention data reported in the literature (Piasenzotto et al., 2003) allowed confirmation of the identity of this compound as benzeneacetonitrile.

3.2. Occurrence in honeys

The range of relative concentrations (percentage of total volatile content) estimated from TIC peak areas for nitriles A–I is shown in Table 1. Nitriles F and I were present at high relative concentrations, compound F usually being

Table 1
Identification data and range for relative concentration (percentage of total volatile composition) of nitriles A–I in *Taraxacum* honeys

Code	RI[a]	RI[b]	Mass spectra (*m/z* (%))	Compound	Identification	Range (%)
A	1010	1009	42 (28%), 68 (20%), 54 (8%)	2-Methylpropanenitrile	RI[a,b], MS	0.26–0.99
B	1091	1094	55 (52%), 54 (25%), 41 (4%), 39 (4%), 68 (1%), 82 (1%)	2-Methylbutanenitrile	RI[a,b], MS, commercial standard	0.47–3.59
C	1134	1131	43 (43%), 41 (27%), 39 (8%), 68 (3%), 55 (1%), 82 (1%)	3-Methylbutanenitrile	RI[a,b], MS, commercial standard	2.62–18.29
D	1162	1067[c]	41 (27%), 67 (19%), 39 (13%), 52 (3%)	2-Butenenitrile (isomer not identified)	RI[a], MS, commercial standard (mixture of *cis*- and *trans*-isomers)	0–0.54
E	1186	1085[c]	41 (29%), 67 (14%), 39 (13%), 52 (4%)	3-Butenenitrile	MS, elution order	0–0.35
F	1241	1241	57 (40%), 41 (21%), 39 (6%), 68 (3%), 82 (1%), 96 (1%)	3-Methylpentanenitrile	RI[a,b], MS, synthesized standard	9.67–33.87
G	1253	–	55 (31%), 41 (14%), 43 (13%), 54 (9%), 57 (7%), 82 (3%), 68 (1%), 96 (1%)	4-Methylpentanenitrile	MS	<0.01–0.20
H	1629	1553[d]	103 (40%), 76 (13%), 50 (5%)	Benzonitrile	MS	0.01–0.05
I	1954	1947	117 (30%), 90 (14%), 63 (3%), 51 (3%), 77 (2%)	Benzeneacetonitrile	RI[a,b] MS	0.82–11.19

RI, retention index and MS, mass spectra.
[a] Experimental data.
[b] Data from the literature (see text for references).
[c] RI at 50 °C
[d] RI at 125 °C.

the main peak in chromatograms of *Taraxacum* honeys analysed.

In order to confirm *Taraxacum* sp. flowers as the source of nitriles, these were submitted to two different extraction procedures: headspace SPME and extraction with acetone. TIC chromatograms obtained by both approaches showed no signal for any of the nitriles above identified.

Nitriles could also arise from nectar of other plants contributing to these honeys. *Taraxacum* sp. are herbaceous plants which blossom from February to May. Plants with similar blossom time could then supply the nitriles detected in *Taraxacum* labelled honeys. Melissopalynological analysis of *Taraxacum* honey showed the presence in similar proportions (approx. 30% each) of *Diplotaxis* sp. and *Taraxacum* sp. pollen. Other pollen contributions, detected in lower proportion, were *Cistus* sp., *Rosmarinus* sp., *Echium* sp., *Salix* sp. and *Prunus* sp. (approx. 12%).

According to the International Honey Commission, *Taraxacum* pollen rarely exceeds 50% in *Taraxacum* labelled honeys, the contributions of other associated plants, such as from *Salix* or *Brassicaceae*, being in higher proportions (Persano Oddo & Piro, 2005). *Brassicaceae* plants, such as *Diplotaxis*, *Brassica* and others have been reported to contain glucosinolates (Daxenbichler et al., 1991). Hydrolysis of glucosinolates gives rise to nitriles and other products (Bones & Rossiter, 1996) which have been found in the *Diplotaxis* genus (Rodriguez, Vela Gurovic, Mulet, & Murray, 2006).

Thus, it would be possible that nitriles appearing in *Taraxacum* honeys were formed from glucosinolates supplied by *Brassicaceae* flowers. This is in agreement with Radovic et al. (2001) who reported the presence of 2-methylpropanenitrile as a characteristic of *Brassica* honeys, and this also could explain the presence of nitriles in other honeys from plants flowering in early spring, such as *Rosmarinus* and *E. japonica* (loquat) honey (De la Fuente et al., 2007).

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