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Characterization of the volatile profile of thistle honey using headspace solid-phase microextraction and gas chromatography–mass spectrometry

F. Bianchi, A. Mangia, M. Mattarozzi, M. Musci *

Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Università degli Studi di Parma, Parco Area delle Scienze 17/A, 43124 Parma, Italy

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ABSTRACT

In this study, a headspace solid-phase microextraction method was developed for the characterization of the volatile fraction of thistle honey and compared with a dynamic headspace extraction method. A DVB/CAR/PDMS fibre was used. The effects of extraction time, equilibration time and salt addition on extraction yield were evaluated. The volatile fraction of seven Italian thistle honey samples was extracted under the optimized conditions and analyzed by gas chromatography–mass spectrometry. Characterization of the volatile profile was performed in terms of nature and relative amount of the extracted compounds. A total of 40 compounds, belonging to different chemical classes, were identified. The relative amounts of 16 compounds found in all the analyzed thistle honeys, i.e. nonanal, furfural, decanal, 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran, benzaldehyde, α-linalool, lilac aldehyde (isomer IV), hotrienol, phenylacetaldehyde, 4-oxoisophorone, benzyl alcohol, 2-phenylethanol, a *not identified* compound, octanoic acid, nonanoic acid and methyl anthranilate, were calculated and submitted to statistical analysis, in order to define for each compound a typical range. On the basis of the obtained data, a characteristic set of values was defined for thistle honey volatile fingerprint. The developed model proved to be effective in recognizing the botanical origin of thistle honey.

1. Introduction

The assessment of the authenticity of honey in terms of its botanical origin is a subject of primary importance for both consumers and industries (Arvanitoyannis, Chalhoub, Gotsiou, Lydakis-Simantiris, & Kefalas, 2005). In fact, consumers demand for a product whose characteristics correspond to those claimed in the label. On the other hand, product authentication is essential from an economical point of view in order to avoid unfair competition. According to the European legislation, honey labelling should provide information also on floral origin (Council Directive 2001/110/EC, 2002).

Usually, the determination of the botanical origin of honey is carried out by melissopalynological analysis, based on the identification of pollen by microscopic examination (Louveaux, Maurizio, & Vorwohl, 1978). However, this technique presents several drawbacks, mainly due to the high skillfulness requested for data interpretation; in addition, it sometimes fails especially when a low amount of pollen is present in the honey.

For these reasons, great attention has been paid in recent years to the development of alternative analytical methods for the assessment of the botanical origin of honey (Arvanitoyannis et al., 2005; Cuevas-Glory, Pino, Santiago, & Sauri-Duch, 2007; Lolli, Bertelli, Plessi, Sabatini, & Restani, 2008; Tomás-Barberán, Martos, Ferreres, Radovich, & Anklam, 2001). Among these alternative approaches, the characterization of the volatile profile has proven to be effective for the assessment of the honey botanical origin; in fact, the volatile profile represents a chemical fingerprint of honey of different botanical origin, since both the nature and the amount of volatile compounds depend on the floral source (Aliferis, Tarantilis, Harizanis, & Alissandrakis, 2010; Baroni et al., 2006; Cuevas-Glory et al., 2007).

Solid-phase microextraction (SPME) is a rapid, solvent free and easy-to-use extraction technique widely applied in the determination of volatile compounds in several foods (Kataoka, Lord, & Pawliszyn, 2000). With respect to other gas-phase extraction techniques, such as dynamic headspace (DHS), already adopted for determination of volatile compounds in honey (Radovic et al., 2001; Soria, Martinez-Castro, & Sanz, 2008), SPME does not require a special and expensive instrumentation and allows the simultaneous extraction of compounds within a wide range of volatility and polarity. In the past years, SPME has been used in several studies aimed to characterize the volatile fraction of honey (Alissandrakis, Tarantalis, Harizanis, & Polissiou, 2007; Ampuero, Bodganov, & Bosset, 2004; Bertelli, Papotti, Lolli, Sabatini, & Plessi, 2008; Cuevas-Glory et al., 2007; Pontes, Marques, & Câmara, 2007; Soria, Sanz, & Martinez-Castro, 2009; Zhou, Wintersteen, & Cadwallader, 2002).

* Corresponding author. Tel.: +39 0521 905128; fax: +39 0521 905555.
E-mail address: marilena.musci@unipr.it (M. Musci).

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In Italy, thistle honey is produced only in three regions, namely Sardegna, Sicilia and Calabria, from floral species belonging to the Compositae family, mainly *Galactites tomentosa*, growing in uncultivated Mediterranean lands and flowering from May to July (Persano Oddo et al., 2000). Thistle honey colour is amber with green tints; the odour is intense and the taste slightly bitter. Its production is very poor, so it can be considered as a rare honey; for this reason and for its organoleptic properties, which are greatly appreciated from consumers, its price is significantly higher than that of other monofloral honeys. The assessment of the authenticity of thistle honey is made difficult because of the low pollen percentage (5–25%); in addition, the limited production and the contribution of different botanical species do not allow defining reliable melissopalynological reference values.

In this work the volatile fraction of thistle honey was analyzed by means of headspace (HS) SPME coupled with gas chromatography–mass spectrometry (GC–MS). To our knowledge, only one study has been previously carried out dealing with the characterization of the volatile profile of thistle honey from New Zealand (Wilkins, Lu, & Tan, 1993), where the volatile compounds have been extracted by using diethyl ether and analyzed by GC-FID.

2. Materials and methods

2.1. Honey samples

Seven samples of thistle honey (Th1–Th7) were obtained directly from the beekeepers. The floral origin was defined by the producers on the basis of the beehives location, season, availability of the floral source and organoleptic characteristics. A sample of multifloral honey (Mf) was also analyzed. The honey samples were stored in hermetically closed glass bottles at 4 °C until analysis.

2.2. Solid-phase microextraction

Preliminary experiments were carried out in order to compare the performances of the DHS extraction technique with those of HS-SPME. Both extractions were performed on the same honey sample (Th4). DHS was performed following the procedure described in a previous study dealing with the characterization of the volatile profile of honey (Radovic et al., 2001). Tree replicated DHS extractions were performed for each sample.

HS-SPME was performed using a 2 cm – 50/30 μm DVB/CAR/PDMS fibre (Supelco, Bellefonte, CA, USA). Before analysis, fibre was preconditioned in the injector of the gas chromatograph at 270 °C for 1 h. Samples were prepared for HS-SPME by mixing 5 g of honey with 5 ml of bidistilled water in 20 ml amber vials hermetically closed with PTFE/silicon septa. The SPME fibre was then exposed to the headspace of the sample. During the equilibration step (20 min) and the extraction step (40 min), the vial was maintained at 60 °C under magnetic stirring. After sampling, the extracted volatile compounds were thermally desorbed at 250 °C for 2 min (splitless mode). HS-SPME analyses were performed under the optimized conditions: extraction temperature 60 °C; extraction time 40 min, salt addition (NaCl) 30% (w/w); magnetic stirring. Tree replicated analyses were performed for each sample.

2.3. Gas chromatography–mass spectrometry

The GC–MS analysis was performed by using a TRACE GC 2000 gas chromatograph (Thermo Electron Corporation, Walthan, MA, USA) equipped with a Finningan TRACE MS mass spectrometer (Thermo Electron Corporation). Helium was used as the carrier gas at a flow rate of 1 ml min^{-1}. The chromatographic separation was performed on a 30 m × 0.25 mm, d_f 0.25 μm Supelcowax-10™ capillary column (Supelco, Palo Alto, CA, USA). The following GC oven temperature program was applied: 35 °C for 8 min, 6 °C min^{-1} to 60 °C, 4 °C min^{-1} to 160 °C, 20 °C min^{-1} to 200 °C, 200 °C hold for 1 min. The transfer line and source were maintained at the temperature of 250 °C and 230 °C, respectively. Electron impact mass spectra were recorded at 70 eV ionization energy by scanning the mass spectrometer from 35 to 300 *m/z* (scan time, 0.5 s). Signal acquisition and data processing were performed using the Excalibur V 1.2 (Thermo Electron Corporation).

The identification of the volatile compounds was performed by comparing the obtained mass spectra with those stored in the National Institute of Standards and Technology (NIST) US Government library. In addition, retention indices (RIs) were calculated for each peak and compared with literature data (Bianchi, Careri, Mangia, & Musci, 2007; Soria et al., 2009 and references therein). In order to evaluate the semi-quantitative differences in the aromatic profile of the samples investigated, the gas chromatographic peak areas were calculated as total ion current (TIC).

2.4. Statistical analysis

In order to maximize extraction recovery, the effects of equilibration time, extraction time and salt addition on SPME recovery were evaluated by submitting the chromatographic peak areas, obtained by operating under different extraction conditions, to one-way analysis of variance (ANOVA), followed by a Bonferroni post hoc test. A p value less than 0.05 was considered statistically significant.

In order to define a set of relative amounts of the volatile compounds distinctive for thistle honey, the chromatographic peak areas of the compounds found in all the analyzed thistle honey samples were elaborated following the approach proposed by the International Honey Commission for the classification of European unifloral honeys (Persano Oddo & Piro, 2004). Firstly, for each sample, the peak areas were normalized with respect to the hotrienol area, dividing, for each honey sample, the area of each peak by the area of the hotrienol. Then, for each compound, the mean and standard deviation of the normalized areas were calculated over the whole data set, thus defining for each compound a reference interval. The predictive ability of the model was then evaluated by the "leave-one-out" cross validation procedure: each sample was removed one-at-time from the initial model, then the model was rebuilt and the sample removed was classified in the new model (Massart et al., 1998). Finally, the calculated model was used to verify the botanical origin of unknown honey samples.

All statistical analyses were performed by using the SPSS package v. 9.0 (SPSS Italia, Bologna, Italy).

3. Results and discussion

3.1. Comparison between DHS and HS-SPME extraction

Representative volatile profiles of the same thistle honey sample (Th4) extracted by DHS and HS-SPME are shown in Fig. 1. The chromatograms were compared in terms of number of extracted compounds and peak areas (Table 1). A total of 53 volatile compounds were extracted, among which 24 were extracted by means of DHS, whereas HS-SPME allowed the extraction of 40 compounds. Table 1 lists all the extracted compounds according to their elution order. In particular, the most volatile compounds were better extracted by DHS whereas the less volatile compounds were better extracted by HS-SPME. In addition, the highest signal intensity was obtained for the HS-SPME profile, although DHS required a higher amount of sample. Finally, it should be observed that the precision of the HS-SPME was better than that obtained