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Some aspects of dynamic headspace analysis of volatile components in honey

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ABSTRACT

Purge and Trap (P&T) has been evaluated as a fractionation technique for the GC–MS analysis of the most volatile compounds in honey. The volatile fraction of twenty-two commercial honeys of eight different botanical sources (eucalyptus, thyme, citrus, rosemary, heather, lavender, multiflower and honeydew) was characterized by P&T–GC–MS. Hundred volatile compounds were characterized, 18 of them being determined for the first time in honey. Compounds detected included volatiles derived from the floral nectar or honeydew source such as terpenes, furan derivatives from honey processing and storage and other compounds whose origin could be related to microbial or environmental contamination. This method has also been validated with regard to data reproducibility; with relative data (percentage of total volatile composition) showing a better precision over quantitative results calculated using an internal standard. Application of multivariate statistical analysis to P&T–GC–MS data has shown to be very promising to classify samples according to their botanical origin.

1. Introduction

Both honey aroma and its sweet taste are the most important organoleptic properties that determine the selection of this food by consumer. Characterization of honey aroma is therefore a subject of great interest in the apiculture industry, as honeys from different sources (nectar or honeydew) and floral nectars (eucalyptus, rosemary, acacia, etc.) differ in their aroma and taste.

Honey aroma is mainly related to its volatile composition. Gas chromatography coupled to mass spectrometry (GC–MS) combines the high sensitivity and efficacy required for the analysis of the very complex mixtures of volatiles present in honey at low concentrations (Maga, 1983) and provides structural information (mass spectrum) for their qualitative analysis. Although GC–MS has become the technique of choice for characterization of volatile fraction of honey, its application requires a previous fractionation step in which volatiles are isolated from the major components of honey matrix (sugars and water) and preconcentrated.

Several studies have been published on the application of different fractionation techniques to the study of honey volatiles: solvent extraction (SE) (Bicchi, Belliardo, & Frattini, 1983; D'Arcy, Rintoul, Rowland & Blackman, 1997; Rowland, Blackman, D'Arcy & Rintoul, 1995), simultaneous steam distillation–extraction (SDE) (Bicchi et al., 1983; Bonaga & Giumanini, 1986; Alissandrakis, Tarantilis, Harizanis, & Polissiou, 2005), mixed procedures based on SE followed by SDE (Bicchi et al., 1983; Bouseta & Collin, 1995), solid phase extraction (Castro-Vázquez, Pérez-Coello, & Cabezudo, 2003), static headspace (Rowland, Blackman, D'Arcy, & Rintoul, 1995), solid phase dynamic extraction (SPDE) (Ampuero, Bogdanov, & Bosset, 2004) and solid phase microextraction (SPME) (Baroni et al., 2006; de la Fuente, Martínez-Castro, & Sanz, 2005; Piasenzotto, Gracco, & Conte, 2003; Pérez, Sánchez-Brunete, Calvo, & Tadeo, 2002; Soria, González, de Lorenzo, Martínez-Castro, & Sanz, 2004; Soria, Martínez-Castro, & Sanz, 2003; Soria, Sanz, & Martínez-Castro, submitted for publication).

However, few references have been reported on the application of dynamic headspace (Purge and Trap, P&T) (Bianchi, Careri, & Musci, 2005; Bouseta, Collin, & Dufour, 1992; Overton & Manura, 1994; Radovic et al., 2001) for the solvent-free fractionation of honey volatiles. In P&T analysis, volatiles swept by a flow of inert gas are trapped on an adsorbent; thermal desorption of this trap allows volatiles to enter the chromatographic system for separation. This technique affords as main advantages a high sensitivity for fractionation of high-volatility compounds, the absence of extended heating times and the reproducibility associated to a totally automated system (Overton & Manura, 1994).

P&T–GC–MS has been used for characterization of volatile composition of natural and commercial honeys (Overton & Manura, 1994; http://www.sisweb.com/referenc/applnote/app-25-a.htm) and for authentication of honey floral source (Bianchi et al., 2005; Bouseta et al., 1992; Radovic et al., 2001) and geographical location (Tananaki, Thrasyvoulou, Giraudel, & Montury, 2007). The present work was aimed to evaluate and improve several

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aspects of dynamic headspace analysis of honey. Particular emphasis has been paid to data precision with a view to the further application of this methodology for honey characterization.

2. Materials and methods

2.1. Honey samples

Twenty-two commercially available Spanish honeys of different source were selected: 2 rosemary (*Rosmarinus officinalis*), 3 eucalyptus (*Eucalyptus* spp), 4 citrus (*Citrus* spp), 1 thyme (*Thymus* spp), 1 heather (*Ericaceae*), 1 lavender (*Lavandula* spp), 4 honeydew and 6 multiflower. All of them were stored less than 6 months at room temperature until analysis.

2.2. Standards

For selection of the internal standard, a 0.01 mg mL^{-1} solution in pentane (Merck, Darmstadt, Germany) of twenty compounds of different polarity and volatility was used. All standards were of analytical grade and enough purity for GC analysis: *n*-heptanal, benzaldehyde and 5-nonanone (PolyScience Corp., Niles, IL, USA); 1-hexanol, nerol and coumarine (Sigma Chemical Co., St. Louis, MO, USA); β-myrcene, terpinen-4-ol, 2-dodecanone and carvacrol (Fluka Chemie, Buchs, Switzerland); octane, nonane and 2-decanone (Merck, Darmstadt, Germany); acetophenone (Analyticals Carlo Erba); phenylacetaldehyde, 2-phenylethanol, geraniol, 2-aminoacetophenone, camphor and methyl anthranilate (Aldrich, Sigma–Aldrich Chemie, Steinheim, Germany).

n-Alkane mixtures (from decane to eicosane) in heptane (Merck, Darmstadt, Germany) were employed for linear retention index (LRI) calculations.

2.3. P&T–GC–MS analysis

Analysis of honey volatiles was carried out using a HP-7695 Purge and Trap concentrator coupled on line to a HP-5890 gas chromatograph with a HP-5971 quadrupole mass detector (Hewlett-Packard, Palo Alto, CA, USA).

Different honey solutions in Milli-Q water (*w*/*v*: 1:5, 5:2, 5:5, 5:18, 8:5) were weighted into a 5 mL P&T fritless vessel. After spiking 10 μL of 0.1 mg mL^{-1} 5-nonanone as internal standard, the vessel was heated at the purge temperature (T_p = 25, 60 or 80 °C) for the time of purge (t_p = 15, 30, 45 or 60 min) using a 37.5 mL min^{-1} helium flow. Volatiles swept by the helium flow and collected at ambient temperature on a Tenax TA trap (Supelco Inc., Bellefonte, PA, USA) were thermally desorbed at 220 °C for 5 min and then concentrated in a narrow band at the end of a fused silica capillary transfer line, indirectly cooled with liquid nitrogen. Cryo-injection (from −100 to 200 °C in 2 min) allowed volatiles to enter the injection port of the GC–MS system. The capillary transfer line and valves were heated at 200 °C in order to avoid volatile compound condensation. Complete control of cooling–heating temperatures and timing was automatically performed by a PC.

Chromatographic separations were carried out on a Supelcowax™-10 capillary column (50 m × 0.25 mm I.D. × 0.25 μm film thickness) (Supelco Inc., Bellefonte, PA, USA). The oven temperature was kept at 45 °C for 15 min, temperature programmed from 45 to 75 at 3 °C min^{-1} and then to 180 °C at 5 °C min^{-1}, remaining at the maximum temperature for 10 min. He at ~1 mL min^{-1} was used as carrier gas. P&T–GC–MS blank analyses were run between consecutive analyses.

Mass spectra were recorded in electron impact (EI) mode at 70 eV, scanning the 35–450 *m/z* range. Interface and source temperature were 280 °C and 230 °C, respectively. Data acquisition and data processing were carried out by using the HP G1034 C v.3.00 MS ChemStation software.

2.4. Qualitative and quantitative determination

Qualitative analysis was based on the comparison of the obtained spectra with those of the Wiley mass spectral library (McLafferty & Stauffe, 1989) and was confirmed by using linear retention indices (LRI). When identification was not possible, elemental composition has been given if it could be deduced from the mass spectrum of the peak. Otherwise, peak retention index and the *m/z* values of the most important ions, along with their abundances relative to the most intense ion, are given for peak characterization.

Concentration data (ng g^{-1} of honey) were calculated by using 5-nonanone as internal standard and relative data (percentage of total volatile composition) were directly obtained from total ion current (TIC) peak areas.

2.5. Statistical data analysis

Principal component analysis (PCA) and stepwise discriminant analysis (SDA) of relative data were carried out by using the 4M and 7M programs, respectively, in the BMDP software for PC computers (BMDP Statistical Software release 7 & Los Angeles, 1992).

3. Results and discussion

3.1. Selection of operating conditions

Concentration of the honey solutions and temperature (T_p) and time (t_p) of purge were optimized to maximize the amount of volatiles extracted, but artifact formation and analysis time were also taken into account.

First, three honey concentrations (1, 5 and 8 g of honey dissolved into 5 mL of Milli-Q water) were tested to select the best quantitative response for most volatiles in the P&T–GC–MS chromatograms. The 5:5 (*w*:*v*) solution was selected as optimal as it simultaneously provided enough sensitivity for medium volatility compounds and did not give rise to saturated peaks for the high-volatility compounds more efficiently extracted by this technique. The influence of the viscosity of the honey solution (*w*/*v*: 5:2, 5:5 and 5:18) on the purge efficiency was also evaluated. As no significant change with honey dilution was observed in the total volatile amount extracted, the most concentrated honey solution was chosen to avoid splashing as a possible source of contamination of the system. Addition of 1-tetradecanol as antifoaming agent and the use of fritless vessels were also found to be advisable for this purpose.

TIC profiles collected at a purge temperature of 25, 60 and 80 °C and a constant purge time of 15 min showed a higher recovery in all the volatility range when increasing T_p. A significant improvement in the fractionation at 80 °C of low-volatility compounds was obtained when raising t_p from 15 to 60 min.

Fig. 1 shows the TIC profiles obtained after three consecutive extractions on the same honey solution (t_p = 15, 30 and 45 min) at a purge temperature of 80 °C. It has been described that heating of honey above ambient temperature for an extended period of time can originate its thermal degradation, giving rise to an increase in the absolute amount of certain heating markers (Singh & Bath, 1997, 1998; Villamiel, del Castillo, Corzo, & Olano, 2001; Visser, Allen, & Shaw, 1988). This effect, which was not very significant in honeys heated at 80 °C for 15 min, considerably raised the concentration of several compounds such as furfural (peak labelled with "*" in Fig. 1) for purge times higher than 45 min, regardless of