Assessment of gas chromatography time-of-flight accurate mass spectrometry for identification of volatile and semi-volatile compounds in honey

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ABSTRACT

The performance of gas chromatography (GC) combined with a hybrid quadrupole time-of-flight (QTOF) mass spectrometry (MS) system for the determination of volatile and semi-volatile compounds in honey samples is evaluated. After headspace (HS) solid-phase microextraction (SPME) of samples, the accurate mass capabilities of the above system were evaluated for compounds identification. Accurate scan electron impact (EI) MS spectra allowed discriminating compounds displaying the same nominal masses, but having different empirical formulae. Moreover, the use of a mass window with a width of 0.005 Da provided highly specific chromatograms for selected ions, avoiding the contribution of interferences to their peak areas. Additional information derived from positive chemical ionization (PCI) MS spectra and ion product scan MS/MS spectra permitted confirming the identity of novel compounds. The above possibilities are illustrated with examples of honey aroma compounds, belonging to different chemical classes and containing different elements in their molecules. Examples of compounds whose structures could not be described are also provided. Overall, 84 compounds, from a total of 89 species, could be identified in 19 honey samples from 3 different geographic areas in the world. The suitability of responses measured for selected ions, corresponding to above species, for authentication purposes is assessed through principal components analysis.

1. Introduction

Honey is a complex natural product which is largely consumed worldwide. It is elaborated by honey bees from carbohydrate-containing exudates produced by plants. In addition to sugars, honey contains moisture and other valuable nutrients such as vitamins, minerals, enzymes, free amino acids and numerous volatile as well as semi-volatile species [1,2]. The two latter groups of compounds are responsible for the aromatic profile, which is one of the most distinctive features of honey [1]. Thus, profiles of volatile compounds can be used for honey authentication purposes, since they tend to differ based on the floral origin as well as the processing type [3–5]. Furthermore, volatile and semi-volatile compounds can be also correlated with antimicrobial and other medicinal properties of honey. Consequently, many efforts, in the

field of sample preparation and analytes determination, have been conducted for the characterization and the identification of new compounds in this foodstuff. Even though gas chromatography combined with mass spectrometry (GC–MS) is the reference technique for volatile and semi-volatile compounds analysis [6], the obtained information is conditioned by several parameters, such as the sample preparation technique, the efficiency and selectivity of the capillary column, and the particular features of the different types of mass analyzers usually implemented in bench-top GC–MS instruments.

With regards to sample preparation, purge and trap (P&T) and headspace (HS) solid-phase microextraction (SPME) are the preferred techniques for the concentration of honey volatiles [7–11]. Nowadays, it is recognized that HS-SPME covers a higher number of species than P&T (particularly semi-volatile compounds), it is more versatile because different fiber coatings are commercially available, and it does not require any modification in the GC instrument at the level of sample introduction. As regards to the type of column, semi-polar and polar coatings offer (1) a higher

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selectivity than non-polar ones, reducing the number of co-eluted peaks; and (2) maximum operational temperatures high enough to deal with species concentrated by HS-SPME [12–14].

The differences between the information provided by MS spectrometers are related to their sensitivity and mass resolution. Quadrupole instruments present a limited sensitivity when operated in the scan mode; therefore, they are unsuitable for minor compounds (or those displaying a limited affinity for the SPME fiber) detection. Ion-trap (IT) and time-of-flight (TOF) MS systems provide an enhanced sensitivity for the screening of unknown compounds. Furthermore, the latter offers mass resolutions in the range from 3500 to 8000 for ions with m/z ratios from 69 to 500 units. High resolution MS spectra could be useful for the identification of novel compounds in honey samples and/or for discerning between species with the same nominal mass, but having different empirical formulae. The suitability of hybrid QTOF systems, providing accurate MS and MS/MS spectral information, for the screening of anthropogenic compounds (e.g. pesticides) in foodstuff vegetable samples [15–17] and antimicrobial, volatile species in medicinal plants [18,19] has been already demonstrated. However, their applicability for honey aroma compounds identification has not been assessed yet.

Multivariate analysis strategies (i.e. principal component analysis, PCA) employed to investigate the authenticity and origin of honey, usually deal with profiles corresponding to total ion current (TIC) GC–MS chromatograms. However, due to the complexity of these chromatograms, the possibility of having several compounds under the same peak is not negligible, particularly, when chromatographic deconvolution is not performed. This fact introduces an additional variability which turns more difficult honey classification. The selection of responses for specific ions, isolated with mass windows in the region of the low milidaltons (mDa), limits the risk that signals are the contribution of several compounds; thus, they could be employed for classification purposes, even when the molecular species responsible for the selected ion has not been identified.

The aim of this study is to assess the possibilities of GC–MS, based on the use of a hybrid QTOF instrument, for the identification of volatile and semi-volatile compounds in honey samples from three different geographical areas of the world, using single MS, tandem MS/MS and different ionization modes capabilities of that system. HS-SPME was considered as an extraction technique, adapting previously reported sample preparation conditions. Responses measured for characteristic ions were employed to discriminate the three groups of samples, attending to their geographic origins and independently of their mono- or multi-floral character. Possibilities, and limitations, of the GC–QTOF–MS for the unambiguous identification of chromatographic peaks are discussed by selecting compounds from different chemical families. Also, the presence of new compounds in honey is reported.

2. Experimental

2.1. Chemicals and reagents

Standards of 1,4-dichlorobenzene, toluene, 4-quinolinecarboxaldehyde, 3-quinolinecarbonitrile, 1-isoquinolinecarbonitrile, 2,4,6-trichlorobiphenyl (PCB-30) and 3-octanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). The latter two species were considered as possible internal surrogates (IS) during HS-SPME, although only 3-octanol was used under the final conditions. Individual stock solutions of the above compounds were prepared in acetone. Further dilutions were made in the same solvent (case of IS), and in ethyl acetate for direct injection of the resulting standards in the GC–QTOF–MS system. A mixture of n-alkanes (C_8–C_{40}) in dichloromethane was provided by Supelco (Bellefonte, PA, USA). This solution was employed to calculate the linear retention index (LRI) of compounds identified in honey samples. Ultrapure water was obtained from a Milli-Q Gradient A-10 system (Millipore, Bedford, MA, USA).

A manual SPME holder, poly(dimethylsiloxane)–divinylbenzene (PDMS/DVB, 65 µm film thickness, 1 cm length) and DVB–carboxen–PDMS (DVB/CAR/PDMS, 50/30 µm film thickness, 2 cm length) coated fibers were obtained from Supelco (Bellefone, PA, USA). Before being used for the first time, fibers were thermally conditioned at the temperatures recommended by the supplier.

2.2. Samples and sample preparation conditions

A total of 19 samples, obtained in three different geographic areas of the world (Galician: Northwest of Spain, Malaysia and Bangladesh) and corresponding to mono- and multi-floral honeys, were employed in this work. Samples were provided by bee keepers and the respective Ministries of Agriculture (case of some honeys from Malaysia and Bangladesh). Codes corresponding to each sample and information regarding the collection year, and their mono- or multi-floral character are summarized in Table 1.

Table 1
Characteristics and geographic origin of honey samples.

Code	Type	Region	Color	Harvest year
GAL-8	Multi-floral	Galician	Light-brown	2012
GAL-14	Multi-floral	Galician	Dark	2012
GAL-15	Multi-floral	Galician	Dark	2012
GAL-16	Multi-floral	Galician	Dark	2012
GAL-17	Multi-floral	Galician	Dark	2012
GAL-18	Multi-floral	Galician	Dark	2012
GAL-19	Multi-floral	Galician	Dark	2012
MY-2	Mono-floral (Pineapple)	Malaysia	Light-brown	2011
MY-3	Mono-floral (Gelam)	Malaysia	Dark	2011
MY-4	Mono-floral (Longan)	Malaysia	Dark	2011
MY-5	Mono-floral (Mangium tree)	Malaysia	Light	2011
MY-6	Mono-floral (Rubber tree)	Malaysia	Light	2011
MY-7	Mono-floral (Sourwood tree)	Malaysia	Dark	2011
MY-8	Multi-floral (Tualang)	Malaysia	Dark	2011
BD-1	Mono-floral (Mustard flower)	Bangladesh	Dark	2012
BD-4	Mono-floral (Kalizira)	Bangladesh	Dark	2012
BD-5	Mono-floral (Padmo flower)	Bangladesh	Light	2012
BD-12	Mono-floral (Mustard flower)	Bangladesh	Dark	2012
BD-21	Multi-floral	Bangladesh	Dark	2012