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# A variety of volatile compounds as markers in Palestinian honey from *Thymus capitatus*, *Thymelaea hirsuta*, and *Tolpis virgata*

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**Abstract**

Three different unifloral Palestinian honey samples that originate from *Thymus capitatus, Thymelaea hirsuta,* and *Tolpis virgata* were analyzed by using Headspace-Solid Phase Microextraction Gas Chromatography–Mass Spectrometry (HS-SPME-GC–MS). The analysis performed exhibited chromatographic profiles that are characteristics for each type of examined honey. Therefore, the proposed analytical procedure demonstrated a rapid characterization method for the analysis of these types of honeys, by revealing the presence or absence of certain organic volatile constituents. A variety of volatiles, particularly phenols, aldehydes, ketones, acids, and alcohols were detected in these types of honeys. The compounds present in each specific honey offer a marker that can be utilized in relating a given honey to its floral origin. *Thymus capitatus* honey showed six marker compounds: 1,3-diphenyl-2-propanone, (3-methylbutyl)benzene, 3,4,5-trimethoxy benzaldehyde, 3,4-dimethoxy benzaldehyde, vanilline, and thymol. *Thymelaea hirsuta* honey is characterized by the presence of a group of alcohols and phenols particularly benzene propanol, benzylalcohol, nonanol, hexanol and 4-methoxyphenol. *Tolpis virgata* hooney, however, has two marker compounds 3,5-dihydroxytoluene, and tridecane.


*Keywords:* Honey; Volatiles; *Thymus capitatus*; *Thymelaea hirsute*; *Tolpis virgata*

## 1. Introduction

Honey is considered as a food that promotes good health since ancient times (Sheppard, Shoukry, & Kamel, 2001). It has long been referred to as the "nectar of the gods". Honey is one of the products that have been mentioned in almost all the ancient mythological texts (Aparna & Rajalakshmi, 1999; Crane, 2004; Hussein, 2000). The importance of honey for the humans is also praised in several classical texts of ancient Greece, such as Homer's Iliad and Odyssey, the Deipnosophists of Athenaeus, and in philosophical texts of Plato, Aristoteles, Democritus, and others (Anderson, Theraulaz, & Deneubourg, 2002; Dumont, 1992; Tsigouri, Passaloglou-Katrali, & Sabatakou, 2004). The fact that Hippocrates, the father of medicine, emphasizes honey for its nutritional and pharmaceutical value is not accidental. It is real that in ancient Greece, honey was used for its nutritional value as well as for medical purposes (Cardetti, 2002; Eaton & Eaton, 2000).

Recently, interest in the analysis of honey has increased with the aim of meeting the regulations requested by different authorities (Al-Mamary, Al-Meeri, & Al-Habori, 2002). Particularly important in this respect is the honey from unifloral origin (Oddo, Piazza, Sabatini, & Accorti, 1995). The botanical origin of honey, which greatly influences consumer preference, remains difficult to determine (Guyot, Scheirman, & Collin, 1999). Therefore, particular stress has been put on the need to develop suitable analytical methodologies to determine the floral origin of honey

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in order to prevent fraud and to authenticate it (Graddon, Morrison, & Smith, 1979). The flavor and aroma of honey vary due to its floral source. In general, light-colored honey is mild in flavor and darker honey has a more pronounced flavor (Castro-Vazquez, Perez-Coello, & Cabezudo, 2003; Schieberle & Komarek, 2003). Flavor/fragrance qualities of honey are very much dependent on the volatile and semi-volatile organic compounds present in both the sample matrix and the headspace aroma (Schieberle & Komarek, 2003). Recently many investigations have been performed on the analysis of volatiles in honey by using different methodologies, all over the world (Barcarolo, Centeleghe, Zanatta, & Cont, 1998; Bonvehi & Coll, 1995; Bouseta, Collin, & Dufour, 1992; Conte et al., 2002; Graddon et al., 1979; Guidotti & Vitall, 1998; Guyot et al., 1999; Guyot-Declerck, Renson, Bouseta, & Collin, 2002; Kulkami, 1997; Nozal & Bernal, 2002; Perez & Brunete, 2002; Radovic & Carer, 2001; Shimoda, Shigematsu, & Osajima, 1995; Shimoda, Wu, & Osajima, 1996; Song, Fan, & Beaudry, 1998; Steffen & Pawliszyn, 1996; Tan, Holland, Wilkins, & Mc Ghie, 1999; Tomas-Barberan, Martose, Ferreres, Radovic, & Ankalm, 2001; Verzera & Campisi, 1998; Weston, Brocklebank, & Lu, 2000; Zhou, Winterstreen, & Cadwallader, 2002).

Although there are few papers that report about volatiles from *Thymus capitatus* honey (Oddo et al., 1995; Andrade, Ferreres, Gil, & Tomás-Barberán, 1997; Tsigouri et al., 2004; Terrab, Recamales, Hernanz, Francisco, & Heredia, 2004), nothing is mentioned about *Thymelaea hirsuta* and *Tolpis virgata* honeys. The aim of this work is to investigate the presence of volatiles that can be utilized as markers in the Palestinian unifloral honeys specifically in *Thymus capitatus, Thymelaea hirsuta* and, *Tolpis virgata*. The amount of the major volatiles present will be determined quantitatively in each selected type of honey.

## 2. Material and methods

### 2.1. Sample collection

The samples of each type of unifloral honey were obtained from the northern and southern part of Palestine. *Thymus capitatus L.* and *Thymelaea hirsuta L.* were from Hebron district (South) and the *Tolpis virgata* samples were from Tulkarem district (North). Each sample was analyzed three times and the method was proved by repeatability test by determining peak area and retention reproducibility of different classes of compounds. The collected samples were stored directly in a refrigerator at 4 °C.

### 2.2. Reagent

All the standards were purchased from Sigma-Aldrich. Helium was purchased from a local supplier with high-grade purity (99.999%).

### 2.3. Instrumentation

Honey was analyzed using a Shimadzu GC-17A connected to an MS-QP5050A. The GC–MS was operated in the electron impact ionization mode (EI) at 70 eV. The GC was equipped with an Omegawax 250 capillary column (30 m × 0.25 mm × 0.25 μm film thickness) obtained from (Supelco, USA). A SPME microextraction syringe with a 65 μm carbowax/divinylbenzene (CW/DVB) fiber was used for collecting the volatiles and semivolatiles from the headspace of honey samples. A Shimadzu autosampler AOC-20I was used with 2 ml vials sealed with 8 mm double-faced rubber septa and a screw cap with 12 mm hole.

### 2.4. Collection of aroma from the honey sample

Honey (5 g) was put in a 27 ml vial and the latter was sealed with a rubber septum secured with an aluminum cap. The vial was heated to constant temperature in a water path. The SPME fiber was introduced into the vial then removed and disrobed in the injection port of the GC for 5 min.

### 2.5. GC–MS operating conditions

The carrier gas flow was 1.6 ml He/min, column pressure was 100 Kpa. The injector and detector temperatures were 220 °C and 250 °C respectively. The column temperature was held at 60 °C for 1 min, then raised from 60 °C to 200 °C at 10 °C/min and held there for 5 min and from 200 °C to 240 °C at 10 °C /min and held there for 6 min. The program was run in the splitless mode with a mass range of 50–400 u, and the scan interval was 0.5 s. Detector voltage was set at 1.5 kV.

### 2.6. Procedure of the GC–MS analysis

#### 2.6.1. Fiber conditioning

Before use, the fiber was introduced into the injector port for 1 h at 220 °C, and every two runs the fiber was conditioned again for 10 min.

#### 2.6.2. Aroma analysis

The loaded fiber of the SPME was desorbed in the injection port for 5 min at 220 °C.

### 2.7. Peaks identification

The identification of the unknown compounds was based mainly on their retention times in comparison with those of authentic standards, and on comparison of the MS spectra of the volatile compounds separated with those of the NIST/EPA/NIH Mass spectral library (NIST 98).

## 3. Results and discussion

### 3.1. Optimization of the HS-SPME method

An ethanol solution of the volatile standard samples (1 ml) was placed in a 2 ml vial equipped with a septum cap and 1 μl was injected into the GCMS. The injected standards concentrations were 20, 50, 100, 500, and 1000 ng/mL.

To determine the optimal sample equilibration temperature, the analysis was carried out at five different temperatures (40, 50, 60, 70, and 80 °C) at fixed equilibration time.

To select an optimal equilibration exposure time of the SPME fiber, the analysis of honey was equilibrated at 40, 50, 60, 70, and 80 min at fixed temperature.

The analysis of volatiles present in Palestinian honey that originates from *Thymus capitatus, Thymelaea hirsuta,* and *Tolpis virgata* was performed by HS-SPME-GC–MS technique. Since honey is a very complex mixture that contains a high amount of sugar, different parameters were considered in order to optimize the headspace absorption. Among these that have been proven to affect the sensitivity of the SPME technique are the sampling temperature, SPME fiber exposure time, fiber polarity, and matrix weight. We have focused our attention into polar column and fiber to extract polar volatiles from honeys. Upon closely studying our optimized method appears to reveal more polar compounds (alcohol and acid) than other method. We think that this is due to the polarity of both the column (Omegawax) and the fiber (carbowax divinylbenzene), which generate higher system sensitivity to these compounds (Song et al., 1998). Sample temperature equilibration was done using five different temperatures (40, 50, 60, 70, and 80 °C) at fixed time. A decomposition was noticed when the temperature was raised to 80 °C. Because of that, 70 °C and 50 min was chosen as the optimized condition for carrying out the analysis of all the honey's sample under investigation. In order to address the optimized method reproducibility, three successive injections of the same honey were made using identical experimental conditions (sampling time, temperature, and fiber). Relative standard deviation (RST) values of the retention time and peak areas of ten compounds demonstrated excellent reproducibility of 0.02–0.4% for retention time and fairly acceptable values of 1.8–12% for peak area.

### 3.2. Identification of the Palestinian honey constituents

Approximately thirty volatile compounds present in the Palestinian honeys have been detected and identified using HS-SPME-GC–MS analysis. The GC–MS developed method through this investigation provides very good selectivity and sensitivity. A baseline separation was seen almost in all the volatiles separated as shown in the TIC generated (Fig. 1). Among the volatiles detected were compounds that belong to the phenol, ketone, ester, acid and aldehyde families. Fourteen of these compounds were identified. In general they are of low molecular weight, linear and branched aldehydes, phenols and acids.

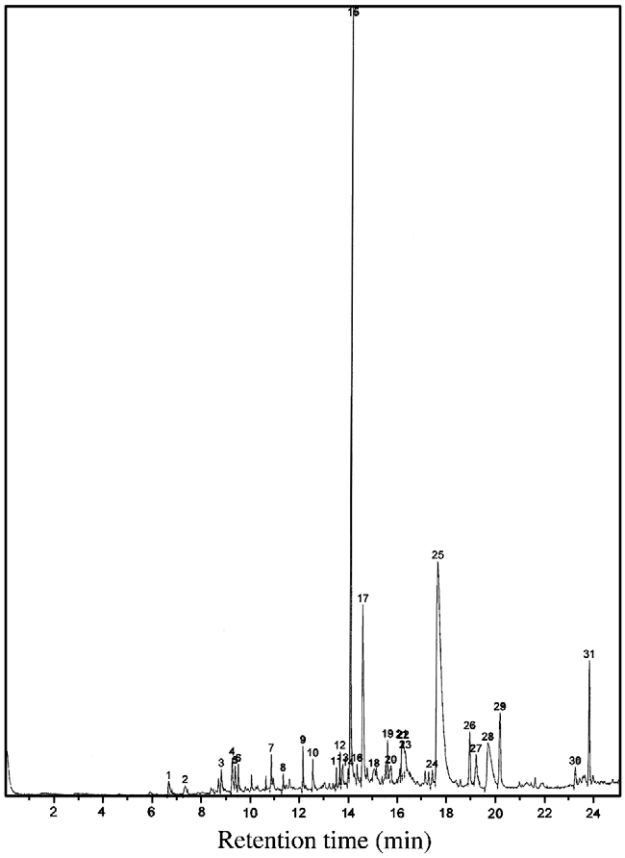


Fig. 1. Total ion chromatogram of *Thymelaea hirsuta* honey. Major peaks identities: 1: hexanol; 2: nonanal; 3: decanal; 4: benzaldehyde; 5: 3,7-dimethyl-1,6-octadien-3-ol; 7: nonanal, 9 – benzyl alcohol; 12: dodecanal; 13: tetradecane 15: phenylethyl alcohol; 17: 2-ethyl hexanoic acid, 19: benzene propanol; 23: propylbenzene; 25: nonanoic acid; 28: decanoic acid; 29: 4-methoxyphenol and 30: 5-hydroxymethyl-2-furancarboxaldehyde.

### 3.3. Determination of the amounts of volatile components in honey

Three replicates of each individual honey samples were analyzed to check the reproducibility of the optimized GC–MS method. The concentration of the unifloral honey volatiles that desorbed from the SPME fiber to the GC was determined by comparing their average peak areas of the sample with a known concentration of the corresponding compounds in the external standard mixture. An external standard curves were prepared for all the standards. The concentrations of the volatile compounds were calculated. The correlation coefficients ($R^2$) values were always >0.99, for all the detected compounds. The HS-SPME-GC–MS method proved to be sensitive as reflected from the limits of detection (LOD) and limits of quantitation (LOQ).

Depending on these results, the following observation can be made: (a) All the samples analyzed contain between two or three carboxylic acids. These acids appear in the

chromatograms as broad peaks as expected; (b) All contain 5-hydroxymethyl-2-furancarboxaldehyde with concentrations higher than 200 ng/5 g in each sample; (c) Two contain benzenacetaldehyde with concentrations 342.3 and 270.7 ng/5 g. In addition two samples contain decanal; (d) All but one contains alcohols in different amount and concentrations ranging between 37 and 978 ng/5 g. Some of these alcohols have a high molecular weight such as phenylethyl alcohol.

### 3.4. Honey markers for the characterization of different unifloral honeys

Our investigation showed that most of the components identified are presents in all honey samples but in different amounts. The ratio between some components of different honey could be used to distinguish the different floral origin.

From the previous results, it is possible to ascertain that the study of volatile fraction of honey using HS-SPME-GC–MS provides useful information for the determination of the unifloral origin of honey. This technique is simple, rapid and fairly reproducible. Fig. 2 summarizes typical proposed markers that could be utilized to characterize the unifloral origin of Palestinian honey.

In general, linear and aromatic aldehydes, short chain alcohols, phenols, and alkanes were found in almost all of the analyzed honey samples.

1,3-diphenyl-2-propanone, 3-methylbutyl benzene, 3,4,5-trimethoxy benzenacetaldehyde, 3,4-dimethoxy benzaldehyde, vanilline, and thymol are compounds that may be used as marker compounds for *Thymus capitatus* honey as shown in Fig. 2A. These volatile compounds are absent in the other two honey type samples and just opposite nonanal and decanal are absent in *Thymus capitatus.*

*Thymelea hirsuta* honey is characterized by the presence of linear chains and aromatic alcohols contrary to the other honey types as Fig. 2B revealed. Six of these compounds are 4-methoxyphenol, benzene propanol, benzylalcohol, nonanol, and hexanol. Therefore, these compounds are markers for this type of honey. In addition, benzeneacetaldehyde, which is present in the other two honey samples, is absent in *Thymelea hirsuta*. Together, the absence and presence of the latter and the former compounds characterize this type of honey.

*Tolpis virgata* has as a marker compounds 3,5-dihydroxytoluene, and tridecane. Propylbenzene, phenylethylalcohol and benzaldehyde, which are present in the other two samples, are absent in this type of honey.

## 4. Conclusions

Due to the presence of different volatile compounds in honey, the HS-SPME-GC–MS technology had been chosen as the analytical method to characterize the origin of unifloral Palestinian honey. This method also allows the identification and quantification of all the separated volatiles from honey. The optimal results were obtained using 65 μm carbowax divinylbenzene (DVB), was found to absorb most of the target volatiles than the commonly used polydimethyl siloxane (PDMS) coated fiber. The relative standard deviation, percentage of the retention time and the peak area was an indication of the precision of the proposed method.

In all honey samples the most abundant compounds were aldehydes, organic acids, phenols and alcohols. These compounds are found in different percentages and in different concentrations due to the floral origin of the honey. These results were used to assess certain markers for the types of honey selected. *Thymus capitatus* honey showed six marker compounds; *Thymelea hirsuta* honey with eight ones and *Tolpis virgata* honey with the two ones.

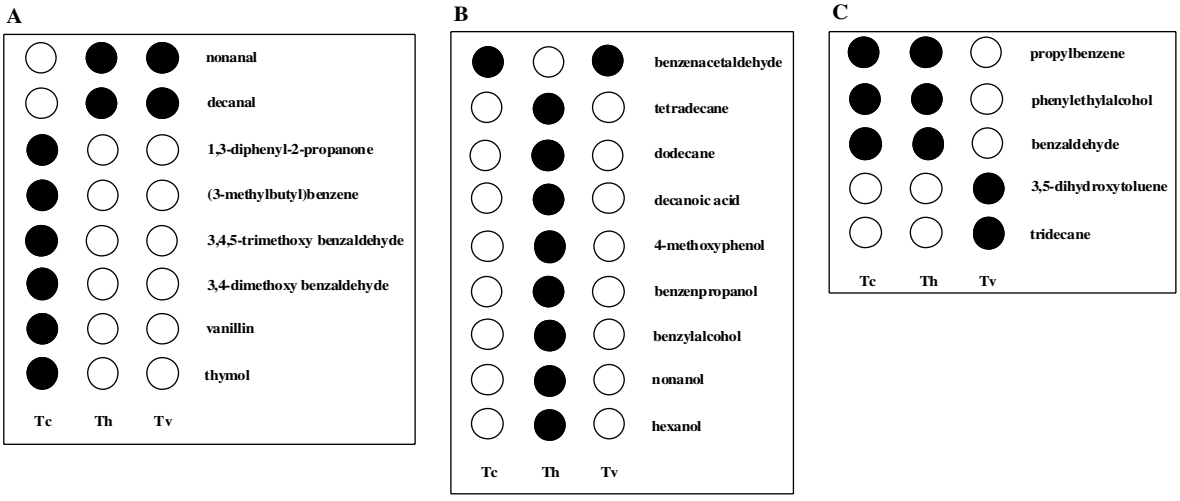


Fig. 2. Marker compounds for differentiation of: (A) *Thymus capitatus* (Tc) honey from *Thymelaea hirsute* (Th) and *Tolpis virata* (Tv) honey. (B) *Th* honey from *Tc* and *Tv* honey and (C) *Tv* honey from *Tc* and *Th* honey. Key: black circles – presence, white circles – absence.

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