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## Determination of volatile compounds of pine honeys

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**Abstract:** The volatile compounds of 13 Turkish pine (*Pinus brutia* Ten.) honey samples were characterized by solid phase microextraction (SPME) analysis, followed by gas chromatography-mass spectrometry (GC-MS) analysis. A total of 42 volatile compounds were identified, but volatile compounds such as nonanal, benzene, 4-hexen-3-ol, alpha-pinene, and 2-heptanone were recognized to be specific floral origin markers of the pine honey. The SPME extraction method was proposed as an alternative way to carry out pollen analysis for floral source detection, especially for products such as pine honey, by characterization of honeydew elements.

**Key words:** Pine honey, volatile compounds, pollen analysis, volatile markers

### Çam balının uçucu bileşiklerinin belirlenmesi

**Özet:** Onüç Türk çam balının uçucu bileşiklerini karakterize etmek için bal örneklerine gaz kromatograf-ütle spektrometrisini takiben katı faz mikroekstraksiyonu uygulandı. Toplam 42 uçucu bileşik tanımlandı; ancak, nonanal, benzen, 4-hekzen-3-ol, alfa pinen ve 2 heptanon çam balının spesifik floral orijin belirteçleri olarak belirlendi. SPME ekstraksiyon metodunun floral kaynak belirlemek için özellikle balçığı elementleriyle karakterize edilen çam balı gibi ballar için polen analizine alternatif olabileceği önerildi.

**Anahtar sözcükler:** Çam balı, uçucu bileşikler, polen analizi, uçucu belirteçleri

### Introduction

Honeydew honey is produced from excretions of plant-sucking insects on the living part of plants. Pine honey is produced only in Turkey and Greece. It is mainly prepared from honeydew secreted by the insect *Marchalina hellenica* (Gennadius), which is restricted to *Pinus brutia* Ten. and *Pinus halepensis* Mill. (1,2). Pine honey is a favored Turkish product owing to its high mineral content.

Several different physicochemical parameters have been used to characterize honeys. Physicochemical characteristics such as pH, acidity, proline content, ash content, color, and electrical conductivity have been considered to be useful characteristics for differentiating floral honeys from honeydew honeys. However, some studies on honeys of different origins and physicochemical characteristics for classification showed that the classification of nectar and honeydew

was not clear (3). The floral sources of honeys have been traditionally authenticated using pollen analysis, which can be considered a reference method at present (4,5). However, this method requires a very experienced analyst; it is time-consuming and difficult to perform.

Since one of the most typical features of honey is its aroma profile, this is used to characterize volatile marker compounds specific to a given botanical origin (6,7). Several studies have been published on the characterization of different floral honeys by their volatile compositions using the following fractionation techniques: solvent extraction (8,9), simultaneous steam distillation-extraction (SDE), dynamic headspace extraction (7,10), and solid phase microextraction (11). However, little attention has been paid to the volatile compositions of honeydew honeys. Owing to the high number of volatile components, the aroma profile represents a "fingerprint" that could be used to determine its botanical origin (7). Solid phase microextraction (SPME) is highly appreciated by the food industry for the analysis of volatile compounds (12). Although honeys of different botanical origin, namely eucalyptus, lime, lavender, heather, and orange, have been previously characterized on the basis of their volatile fractions (10,13,14), to our knowledge, only a limited number of studies regarding the volatile composition of honeydew honeys have been published (15-17). In this study the volatile compositions of 13 Turkish pine (*P. brutia* Ten.) honey samples were extracted using the SPME technique, and the volatile compounds were determined by gas chromatography-mass spectrometry (GC-MS) analysis. Therefore, the aim of this study was to extract the volatile composition of pine honey and determine the volatile compounds as possible markers of the floral sources of the honey.

## Materials and methods

### Honey samples

The 13 pine honeys (*Pinus brutia* Ten.) used in this study were obtained from the Muğla-Marmaris

district of southwestern Turkey. In order to ensure that the honeys were as unifloral as possible, the melissoplagnological procedure was followed (4). Honey samples were characterized on the basis of the ratio between honeydew elements and pollen (HDE:P > 3). All the samples were stored at 4 °C until analysis.

### Determination of volatile compounds

Authentic substances used for identification were from Sigma-Aldrich (Milan, Italy). Analysis of aromatic volatile concentration was performed using a SPME technique (Supelco Co., Bellefonte, PA, USA) and GC (Model 6890; Agilent Technologies, Rockville, MD, USA) for volatile separation and quantification. The isolation of the volatile compounds was performed using the SPME procedure. A polymethylsiloxane fiber was used to extract headspace volatiles from honey. The samples (honey water solution, 5 g/mL) were placed in 15-mL screw-top vials with PTFE/silicone septa. Benzophenone was used as an internal standard, and a portion of 20 µL (10 µg/mL in methanol) was added prior to extraction. The vials were maintained in a water bath during equilibration and extraction, and they were partially submerged so that the liquid phase of the sample could remain under the water.

In order to determine the optimal conditions for the extraction, the following parameters were evaluated: water bath temperature (80 °C), sampling time (30 min), sample volume (5 mL), and salt addition (NaCl and MgSO<sub>4</sub>).

### Analysis of the isolated compounds

The analysis of the extracts was performed using an Agilent 6890 II GC equipped with an Agilent 5973 MS detector. The column used was an HP-5MS (crosslinked 5% phenylmethylsiloxane) capillary column (30 m × 0.25 mm i.d., 0.25 film thickness), and the gas carrier was helium at a rate of 1 mL/min. The injector and MS transfer line temperature were maintained at 250 °C, and the oven temperature was held at 50 °C for 5 min and then raised to 250 °C at 8 °C/min.

Mass spectra were recorded in the electron ionization mode at 70 eV, scanning the 50-550 m/z range. The identification of the isolated compounds was achieved by comparing retention times and mass spectra with those of authentic samples. Identification of volatile components was confirmed by comparison of collected mass spectra with those of standards and spectra in the National Institute for Standards and Technology (NIST) mass spectral library, Search Version 02.00 (Agilent Technologies).

## Results and discussion

The analysis of volatiles present in pine honey was performed with the SPME-GC-MS technique. A total of 42 volatile compounds were identified. Only volatiles occurring in more than 3 samples were reported (Table). Among the volatiles detected were the compounds that belong to the hydrocarbon, ketone, ester, terpene, and organic acid families. Major constituents of the volatile profile of honeydew honeys were nonanal, benzene, 4-hexen-3-ol, alpha-pinene, and 2-heptane. Significant proportions of octane, n-decanal, benzophenone, methyl dihydrojasmonate, and benzeneacetaldehyde were also detected.

Even though nonanoic acid, 2H-benzimidazol-2-one, 2-hydroxymethylbenzimidazole, phosphoric acid, pentanoic acid, and butyric acid are present in relatively higher amounts, they do not aid in botanical discrimination. The possible marker compounds identified are summarized in the Table, and the Figure shows the total ion current GC-MS chromatogram of the volatile fraction obtained by sampling pine honey.

Even though Radovic (7) and other authors have suggested the presence of cis-linaloloxide, heptanal, 2-methyl dihydrofuranone, and alpha-methylbenzyl alcohol in chestnut honey (4); acetoin and 2-pentanone in eucalyptus honey; bicycle-2,2,2,-octan-1-ol-4-methyl and 4-ethylphenyl acetate in heather honey; heptanal in lavender honey; acetone,

2-pentanone, benzaldehyde, and 4-oxoisophorone in rosemary honey; and acetone, furfural, and benzaldehyde in acacia honey, no such compounds were identified in our analyzed pine sample. Some compounds, namely nonanol, nonanal, benzene, alpha-pinene, and 2-heptanone, could be considered markers of this unifloral honey since they were present in all samples analyzed, and they were not detected in other honeys of different botanical origin at all or at levels comparable to those reported by other authors (14). However, these results were consistent with a previous study in which many Greek and Turkish pine honeys were found to have similar compounds, such as toluene, octane, 2-heptanone, nonanal, nonanol, alpha-pinene, benzene acetaldehyde, and benzene (15). In addition, quercitol for *Quercus* honey and aminoacetophenone and propylanisol for holm oak honeydew honeys were found to be good markers for different honeydew honeys (15,18).

According to previous results, it is possible to ascertain that the study of the volatile fraction of honey using SPME-GC-MS provides useful information for the determination of the unifloral or geographic origins of honeys (17). Honeys with the same floral sources can vary due to seasonal climatic variation. Honeydew secretions from pine trees are produced by the same insect (*Marchalina hellenica* L.), but volatile profiles can be different because secondary plants may contribute to volatile profiles.

In the analyzed pine honeys, there were some compounds that were not present in floral honeys. Therefore, it was concluded that the presence or absence of a particular compound in a particular type of honey could be a characteristic feature for a specific type of honey. For this reason, SPME-GC-MS identification of honey headspace volatile components could be useful for the identification of the botanical origin of honey. However, it is necessary to perform more detailed investigations, including the study of a larger number of honey samples from both identical and different botanical sources.

Table. Total ion current GC-MS results obtained by SPME-GC-MS technique for a typical pine honey.

No.	Compounds	RT	Min.	Max.	Avg.	Occurrence
<i>Fatty, carboxylic, and aromatic acids and their esters</i>						
1	Nonanoic acid	14.84	0.47	10.26	4.89	8
2	Hexyl butyrate	16.63	0.04	2.85	1.38	3
3	Butyl butyrate	16.64	1.41	2.65	2.05	3
4	Butyric acid	17.19	8.19	12.2	6.83	3
5	Benzoic acid	17.91	0.48	1.33	0.91	3
6	Methyl salicylate	13.43	1.36	1.46	1.41	3
7	Propanoic acid	16.64	0.58	2.31	1.80	6
8	Phosphoric acid	18.93	4.66	5.27	4.96	3
9	2-Butenedioic acid (fumaric acid)	19.20	0.27	0.39	0.36	5
10	Pentanoic acid	20.12	5.06	7.28	6.34	4
11	Methyl dihydrojasmonate	20.93	0.64	1.16	0.88	9
12	Benzeneacetic acid	21.10	0.6	0.78	0.72	3
13	Hexanedioic acid	21.19	0.25	0.43	0.33	3
14	Thiocyanic acid	23.94	0.42	0.48	0.46	3
<i>Phenolic compounds</i>						
15	Phenol(2,4-bis (1,1-dimethylethyl...))	17.83	0.5	1.58	0.61	4
16	Butylated hydroxyanisole	18.21	0.52	2.46	1.81	3
<i>Hydrocarbons</i>						
17	Toluene	4.54	1.8	2.4	3.02	5
18	Octane	5.24	0.43	1.21	0.84	4
19	Nonanal	11.57	1.69	10.91	6.19	13
20	n-Decanal	13.62	0.81	1.96	1.36	11
21	Cyclotetrasiloxane	14.13	0.35	0.52	0.43	9
22	Benzene	16.29	1.05	11.8	5.83	13
23	Alpha-pinene	16.56	2.03	6.08	4.02	13
24	Cyclohexasiloxane	19.41	0.25	0.59	0.41	5
25	n-Undecane	20.49	0.26	0.46	0.36	5
26	Benzeneacetaldehyde	23.78	0.26	0.84	0.42	4
27	n-Tetracosane	24.12	0.32	0.73	0.41	3
<i>Alcohols, ketones, and terpenes</i>						
28	2-Heptanone	10.3	0.22	0.92	0.46	13
29	Isoborneol	12.86	0.12	0.22	0.15	3
30	Nonanol	12.96	1.45	10.45	5.87	13
31	Damascanone	16.84	2.19	4.04	3.37	3
32	2H-Benzimidazol-2-one	17.14	3.53	6.22	4.88	6
33	4-Hexen-3-ol	18.93	3.01	6.33	4.47	13
34	Benzophenone	20.61	0.33	0.61	0.43	9
35	1-Penten-3-ol	21.44	0.38	0.5	0.43	3
36	Thymol/carvacrol	21.52	0.27	0.30	0.37	5
<i>Others</i>						
37	2(3H)-Benzothiazolone	14.37	1.22	2.34	1.54	3
38	1,3-Benzenediamine	16.82	0.89	1.61	1.32	3
39	2-hydroxymethylbenzimidazole	17.18	8.72	12.01	10.73	3
40	Acetamide	21.54	0.25	0.38	0.29	3
41	Guanidine	23.45	0.12	0.22	0.16	3
42	Dibutyl phthalate	24.95	2.01	5.32	3.21	3

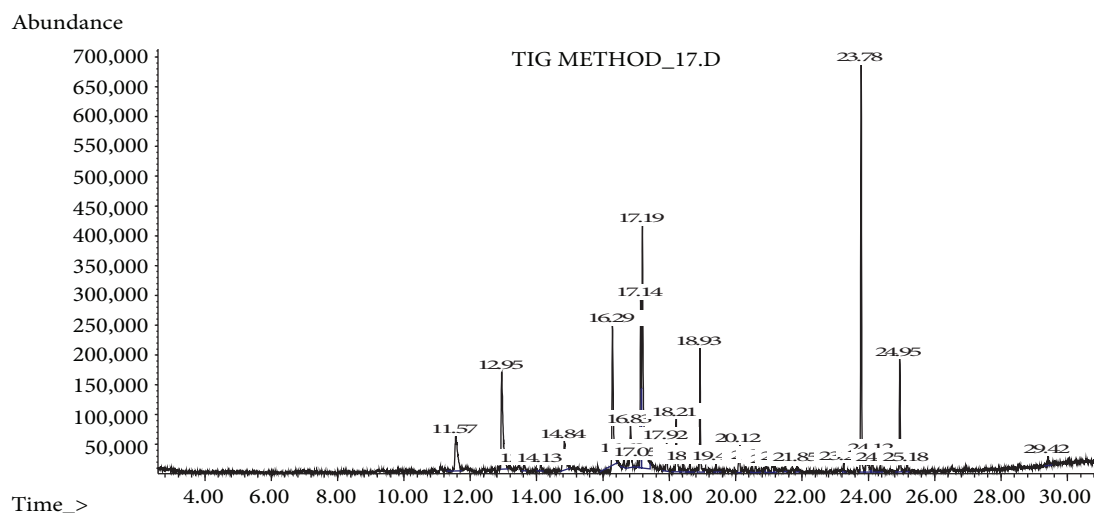


Figure. Volatile profile obtained by SPME-GC MS technique for a typical pine honey.

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