RESEARCH ARTICLE

DETERMINATION OF CHARACTERISTIC VOLATILE COMPOUNDS OF MONOFLORAL CHENOPODIACEAE HONEY BY SPME-GC-MS

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Manuscript Info

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Abstract

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Background: Honey has been used for centuries as a food and for medicinal purposes. For determining the honey origin various analyses are performed, which is classified as blossom honey, secretion honey, and mixed honey.

Methods: The pollen analysis in blossom honeys determines the nectar source and pollen content. The chemical composition and volatile aroma compounds of honey can be determined using the headspace solid phase micro extraction (HS-SPME) method.Volatile compounds are the key factors that determine the aroma, flavor, odor, antioxidant activity, and phenolic content of honey.

Results: In this study, a honey sample provided from Burdur province of Türkiye, was examined using melisopalynological analysis and was identified as monofloral Chenopodiaceae honey. The high predominant pollen content in Chenopodicaeae honey increased the reliability of the identification (95.90%). A total of 32 volatile compounds were detected in the honey sample. 1-Hexanol, 1,5,7-Octatrien-3-ol, 3,7-dimethyl-, 1-Heptanol, and Linalool are the most plentiful marker volatile compounds in Chenopodiaceae honey.

Conclusion: It is thought that monofloral Chenopodiaceae honey, whose authentication was confirmed for the first time by this study, will lead to further research.

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Introduction:

Honey is a sweet, nutritionally valuable animal product produced by honeybees from two different sources. Honeybees produce blossom honeys by collecting flower nectar, and secretion honeys from the secretions of plants or insects living on plants (Bogdanov et al., 2008).In addition to its high carbohydrate content, honey also contains other nutrients such as protein, vitamins, and minerals. The organic acids, phenolic substances, flavonoids, and enzymes in its structure are effective in honey's strong antioxidant properties and in preventing and treating diseases (Al-Mamary et al., 2002; Alvarez-Suarez et al., 2010).The chemical composition and sensory properties of honey vary according to geographical location, vegetation, flora, and climatic conditions. In particular, the taste, smell, aroma, and health effects of blossom honey attached to the nectar source and botanical origin. In this respect, monofloral honeys exhibit specific characteristics (Molan and Cooper, 2000; Noori et al., 2013).Blossom honeys are

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named according to the type of plant source they contain and the percentage of pollen. Honeys that do not have a dominant pollen type are defined as multifloral honey.In normal monofloral honeys, the pollen content must be at least 45% (Melliou and Chinou, 2011).Pollen type and content can be determined through melissopalynological analysis to specify the authentic origin of honey.In addition to pollen analysis, physicochemical or chromatographic techniques can also be used in the identification of honey. Volatile compounds of honey are determined by chromatographic analysis. The volatile compounds affect the aroma and flavor characteristics of honey. The detected specific compounds are useful in the botanical classification of honey and are reported as fingerprints/markers (Escriche et al., 2017; Cuevaz-Glory et al., 2007).Zhu et al. (2022) reported that hexanal, hexanol, heptanol, and methyl enanthate were the discriminatory compounds in the authentication of lavender honey using HS-SPME.

The Chenopodiaceae (goosefoot) family is generally succulent or jointed herbs and shrubs.Most of its species are weedy, and it is a family with few vegetables and field crops.Spinach, chard, sugar beet, quinoa, sea purslane are some of them. Medicinal plants of the Chenopodiaceae family have antidiabetic effects with the bioactive compounds they contain. They stimulate insulin secretion, enhance glucose uptake and inhibit gluconeogenesis (Rubatzky and Yamaguchi, 1997; Cherrada et al., 2024).The Poaceae family is one of the largest plant families. This family, which is the main source of human and animal nutrition, includes grains such as wheat, rice, rye, corn, millet, barley, and oats (Al-hassnwy and HasanAl-nomani, 2021).

Türkiye, with its rich flora, has the potential to produce abundant blossom honey. The most well-known and researched blossom honeys are astragalus, lavender, thyme, chestnut, citrus, sunflower, and black cumin honeys (Kayacier and Karaman, 2008).A total of 95.492 tons of honey was produced in Türkiye in 2024.Burdur province is one of the leading provinces of the Mediterranean Region of Türkiye in the fields of agriculture and animal husbandry.In 2024, 185 tons of honey were produced in Burdur (TUIK, 2025).

In this study, a blossom honey produced in Burdur was identified by determining its pollen content using melissopalynological analysis. The volatile compound profile of the honey sample was characterized by HS-SPME-GC-MS analysis.

Material and Methods:
Honey sample
The honey sample was obtained from a beekeeper in Büğdüz village of Burdur province. The freshly collected honey sample was placed in a dark glass jar and analyzed without waiting.

Melissopalynological analysis
Pollen types and quantities in the honey sample were identified through melisopalynological analysis. To prepare the sample, 10 grams of honey were dissolved in 10 mL of distilled water by heating the mixture to 50 °C. The solution was then centrifuged at 3500 rpm for 45 minutes, after which the supernatant was carefully discarded. The remaining sediment was combined with a glycerin-gelatin matrix and mounted onto a microscope slide. Approximately 300 pollen grains were counted, and the relative frequency of each plant taxon was calculated as a percentage of the total pollen observed.Frequency categories were established in the following way: Dominant pollen over 45%, secondary pollen between 16-45%, significant minor pollen 3-15%, and minute pollen under 3% (Louveaux et al., 1978).

Analysis of volatile compounds
Volatile compounds in the honey sample were analyzed using the HS-SPME method, coupled with a gas chromatograph-mass spectrometer (Agilent 7890B GC, 7010B MS, USA). A total of 3.5 g of honey and 1.1 mL of distilled water were added to a 20 mL headspace vial and incubated at 60 °C for 15 minutes. Volatile compounds were then extracted using a 50/30 µm DVB/CAR/PDMS fiber (1 cm) for 30 minutes. The fiber was exposed to the headspace via a Solid Phase Microextraction (SPME) device.After extraction, the analytes were thermally desorbed for 5 minutes and introduced into a DB-Wax capillary column (60 m x 0.25 mm i.d x 0.25 µm, J&W Scientific-Folsom, USA). The injector temperature was maintained at 250 °C, and the GC oven was programmed as follows: an initial hold at 40 °C for 4 minutes, followed by a ramp of 3 °C/min to 90 °C, then 4 °C/min to 130 °C with a 4-minute hold, and finally 5 °C/min to 240 °C with an 8-minute hold at the final temperature. Helium served as the carrier gas.Mass spectrometry was performed in electron impact (EI) mode with an ionization energy of 70 eV and a scan range of 30-600 m/z. The injection was performed in splitless mode. Quantification of volatiles was carried out using 3-heptanone, 2-methyl- (10 µL) as the internal standard(Xagoraris et al., 2021).

Results and Discussions:

Melissopalynological analysis

The pollen types and percentages were detected by the melissopalynological analysis used in the identification of honey. The results of the analysis were given in Table 1.The honey sample contained a high amount of pollen from the Chenopodiaceae family (95.90%) and being the major pollen, it enables the honey to be defined as monofloral.The honey with two taxa counts, there is 4.10% Poaceae pollen as minor pollen.The palynological origin of honey is strongly influenced by the geographic location, the local flora, and the season or timing of pollen collection.Flora, consisting of plants, wildflowers, fruit trees, vegetable flowers, is a source of raw materials for bees (Can et al., 2015; Bolukbası, 2009).When the flora of the region where the honey was collected was examined, wheat, corn, fruit and vegetable cultivation was identified.In a study examining the pollen analysis of honey from Burdur province, low levels of Chenopodiaceae pollen were found in 7 out of 20 samples (0.015-4.03%).Pollen belonging to the Poaceae family was identified in 11 samples (Taşkın and İnce, 2009).

Table 1: Melissopalynological results of the honey sample.

Predominant pollen (>45%)	Secondary pollen (16-45%)	Important minor pollen (3-15%)	Minor pollen (<3%)	Plant taxa number	Honey type
Chenopodiaceae 95.90%	-	Poaceae 4.10%	-	2	Monofloral Chenopodiaceae honey

Volatile compounds of Chenopodiaceae honey

Volatile compounds, especially in blossom honeys, are trace components that determine the taste, aroma, phenolic content, and antioxidant properties of honey (Cianciosi et al., 2018). The volatile compounds of Chenopodiaceae honey were analyzed by gas chromatography-mass spectroscopy (GC-MS) using the HS-SPME method. The characteristic chromatogram of volatile compounds was given in Figure 1. According to the analysis, 32 volatile compounds were identified in the honey sample. These compounds were listed in Table 2 and ranked from highest to lowest concentration.The identified compound groups include aldehydes, ketones, terpenes, alcohols, and esters.The compound with the highest concentration is 1-Hexanol.Esriche et al. (2017) reported that 1-Hexanol was significantly higher in lavender honey and that this might be a typical marker for lavender honey.Similarly, among monofloral honeys, hexanol concentration was found to be 281 µg/kg in lavender honey, while it was not detected in citrus and heather honeys (Castro-Vazquez et al., 2009).When other alcohols and their amounts are examined (1,5,7-Octatrien-3-ol, 3,7-dimethyl-,1-Heptanol, Linalool, Thymol, Levomenthol, 1-Nonanol, 1-Hexanol, 4-methyl-, (S)-), it can be said that the alcohol group is dominant in this study.According to the volatile compound analysis of quinoa species, a pseudograin from the Chenopodiaceae family, it was revealed that the aldehyde group was more abundant/dominant.A total of 28 distinct volatile compounds were identified across white, red, and black quinoa varieties. The major classes of these compounds included aldehydes (23.99%-36.81%), alcohols (22.65%-26.50%), ketones (12.30%-19.97%), acids (9.69%-10.10%), and esters (13.30%-30.99%), all of which were commonly present in the quinoa samples (Song et al., 2021).
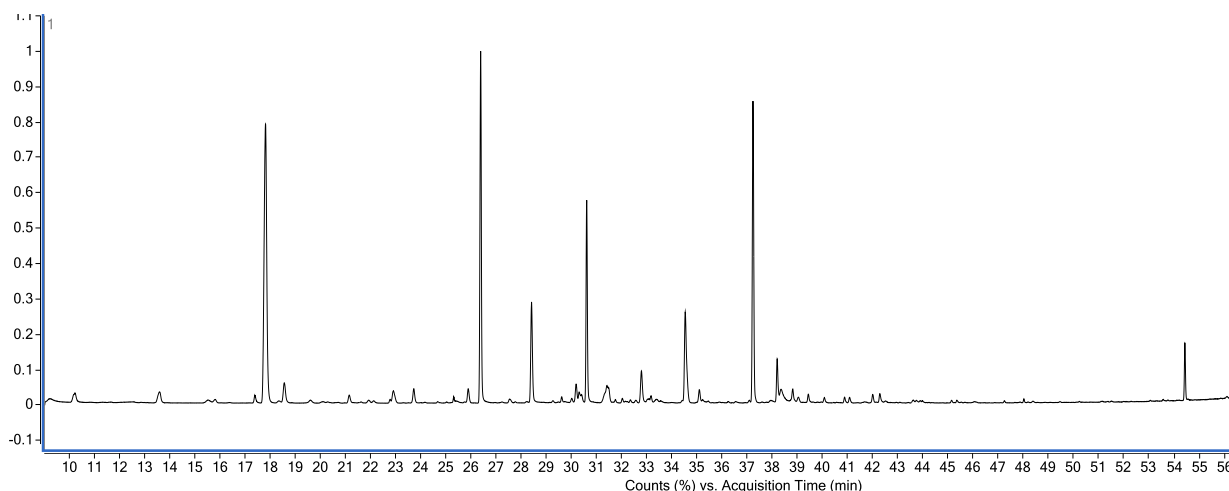

Figure 1: The chromatogram of the volatile compounds of honey.

Table 2: The concentration of the volatile compounds of honey.

	Compounds	Concentration (µg/kg)
1	1-Hexanol	156.920
2	1,5,7-Octatrien-3-ol, 3,7-dimethyl-	138.384
3	1-Heptanol	84.421
4	Linalool	63.504
5	Nonanal	54.526
6	Eicosyl octyl ether	23.775
7	Thymol	19.978
8	Decanal	18.249
9	Heptanal	15.455
10	Hexanal	10.918
11	1,3,5-Cycloheptatriene, 3,7,7-trimethyl-	10.575
12	Octanoic acid, ethyl ester	10.182
13	Octanal	8.530
14	Heptanoic acid, ethyl ester	7.991
15	Benzeneacetaldehyde	7.540
16	trans-Linalool oxide (furanoid)	6.849
17	Octyl chloroformate	6.444
18	Levomenthol	6.295
19	Hexanoic acid, ethyl ester	5.459
20	Oxime-, methoxy-phenyl-_	4.717
21	1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethyl)-	3.923
22	1-Nonanol	3.916
23	Pyrazole, 1,4-dimethyl-	3.432
24	4-(2-Fluorobenzylidene)-2-phenyl-1,3-oxazol-5(4H)-one	3.053
25	endo-Borneol	2.896
26	Dimethyl trisulfide	2.704
27	D-Limonene	2.690
28	2-Cyclohexen-1-one, 4-(1-methylethyl)-	2.667
29	L-.alpha.-Terpineol	2.659
30	Decanoic acid, ethyl ester	2.625
31	1-Hexanol, 4-methyl-, (S)-	2.493
32	3-Hexen-2-one	2.488

The volatile compounds present in honey serve as characteristic indicators of its botanical origin. Studies have shown that honeys have different volatile compounds depending on their species. Some monofloral honeys and their main marker compounds are summarized in Table 3 as a result of the studies.

Table 3: The marker volatile compound/s of different honeys.

Honey type	Marker volatile compound/s	Reference
L. bicolor monofloral honey	Kaempferol-3-*O*-galactoside	Ren et al., 2022
Chestnut honey	Acetophenone,1-phenylethanol, 2-aminoacetophenone	Pattamayutanon et al., 2017
Rhododendron honey	Lilac aldehyde, 2-aminoacetophenone	Senyuva et al., 2009
Citrus honey	3-hydroxy-5-methyl-2-hexanone, methyl anthranilate	Castro-Vazquez et al., 2009
Leatherwood honey	4-Methoxymandelic acid	Moore et al., 2025
Thyme honey	2-phenylacetaldehyde, ethyl nonanoate, benzaldehyde,2-phenylethan-1-ol	Karabagias et al., 2018
Orange honey	Hotrienol (3,7-dimethyl-1,5,7octatrien-3-ol	Verzera et al., 2001
Chestnut honey	acetophenone, 2-aminoacetophenone, and 1-phenylethanol	Guyot et al., 1998
Strawberry tree honey	3,5,5-trimethyl-2-cyclohexen-1-one, 3,5,5-	Bianchi et al., 2005

	trimethyl-3-cyclohexen-1-one, 3,5,5-trimethyl-cyclohex-2-ene-1,4-dione	
Acacia honey	cis-linalool oxide, 3-methyl 3-buten-1-ol, heptanal	Machado et al., 2020

Conclusion:

Honey is an animal product with high bioavailability. While there are many types of honey, interest in specific blossom honeys is quite high. Many properties of honey, such as its chemical composition, plant origin, antioxidant activity, phenolic compounds, and aroma compounds, can be determined through analyses. In this study, a honey sample provided by a beekeeper in Burdur province of Türkiye was examined. Authentic properties of the honey sample were confirmed by melissopalynological analysis. The honey sample, which has two taxa, contained 95.90% Chenopodicaeae pollen and 4.10% Poaceae pollen. The honey was identified as 'Monofloral Chenopodicaeae Honey'. Analysis of the honey sample using the SPME method revealed 32 volatile compounds. The concentration of 1-Hexanol is the highest in monofloral Chenopodicaeae honey (156.920 µg/kg), which contains alcohol, aldehyde, ketone, ester, and terpene compounds. This is followed by 1,5,7-Octatrien-3-ol, 3,7-dimethyl- (138.384 µg/kg), and 1-Heptanol (84.421 µg/kg). These three compounds are considered marker volatile compounds for monofloral Chenopodicaeae honey with their high concentrations. The volatile compounds detected in the honey sample affect the taste, aroma, and bioactivity of honey. Considering factors such as geographical location, flora, and climatic conditions, it can be said that each honey has its own unique characteristics. In this regard, it would be useful to determine the content composition of different honeys and reveal their specific properties. In addition, research on their health effects should be conducted to ensure the effective use of monofloral honeys in apitherapy.

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