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Research Article

Analysis of Volatile Compounds of Some Turkish Flower Honey Samples by Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry

Nur Efşan Durmaz [1*], Rahmi Ertan Anlı [2], Yalçın Güçer [3], Nevzat Artık [4]

[1*] Ankara Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, Ankara, Türkiye (ORCID: 0000-0002-7254-116X), efsandurmaz@gmail.com
[2] Ankara Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, Ankara, Türkiye (ORCID: 0000-0002-3320-0629), anli@eng.ankara.edu.tr
[3] Ankara Üniversitesi, Kalecik Meslek Yüksekokulu, Gıda İşleme Bölümü, Ankara, Türkiye (ORCID: 0000-0002-7280-584X), ygucer@ankara.edu.tr
[4] Ankara Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, Ankara, Türkiye (ORCID: 0000-0001-5583-6719), artik@ankara.edu.tr

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Abstract

Honey is a nutritious food with economic importance for many countries worldwide. It is very important to know the origin of the flower in evaluating the quality of honey, and it is known that honey volatiles play a major role in differentiating different types of honey based on their plant origin. The solid-phase microextraction (SPME) followed by gas chromatography-mass spectrometry (GC/MS) technique is frequently used in the determination of the flower origin of honey through the analysis of volatile aromatic compounds. In this study, five different flower honey provided from different regions of Turkey and some of volatile aroma components which were determined by GC-MS are dl-alanine (% 2.78), acetic acid (% 5.85), butane (% 2.94), furfural (% 12.37), benzaldehyde (% 1.57), benzeneacetaldehyde (% 4.96), benzoic acid (% 2.6), benzoic acid, 2-ethylhexyl ester (% 0.96), ethanol (% 4.22), nonanal (% 1.41), nonanoic acid (% 2.34), hexanoic acid (% 6.47), octanoic acid (% 4.68), methyl 2-furoate (% 3.85), and ethylhexanoic acid (% 3.27). Thus, the effect of different regions on honey aroma was investigated with the aim to reveal honey varieties based on their content of volatile compounds.

Keywords: Honey, Flower, Volatile aromatic compounds, SPME, GC-MS.

Bazı Türk Çiçek Ballarının Uçucu Bileşenlerinin Katı Faz Mikroekstraksiyonu Ve Gaz Kromatografisi-Kütle Spektrometresi İle Analizi

Öz

Bal, dünya çapında birçok ülke için ekonomik öneme sahip besleyici bir gıdadır. Bal kalitesini değerlendirmede çiçeğin kökenini bilmek çok önemlidir ve baldaki uçucu maddelerin, farklı balların bitki orijinlerinden ayrılmasında önemli bir rol oynadığı bilinmektedir. Katı-faz mikro-ekstraksiyonu (SPME) ve gaz kromatografisi-kütle spektrometrisi (GC-MS) tekniği, balın çiçek kökeni tayininde uçucu aromatik bileşiklerin analizi ile sıklıkla kullanılır. Uçucu bileşikler SPME tarafından toplanır ve GC / MS ile analiz edilir. Bu çalışmada, Türkiye'nin farklı bölgelerinden 5 farklı çiçek balının aroma bileşenleri; dl-alanin (% 2.78), asetik asit (% 5.85),

Anahtar Kelimeler: Bal, Çiçek, Uçucu aroma bileşenleri, SPME, GC-MS.

[*] Corresponding Author: Ankara Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, Ankara, Türkiye, ORCID: 0000-0002-7254-116X, efsandurmaz@gmail.com

1. Introduction

Beekeeping provides employment, income, and healthy nutrition for the rural population in developing countries. In recent years, the tendency of the body to provide energy and nutrients from natural sources has been an important factor in the development of apiculture activity in the name of healthy life.

Turkey is a bridge between the Asian and European continents, connects the terms of beekeeping and this location of the country creates an important advantage among world countries in terms of natural wealth. The most known bee product is honey with 99.4% (Kekeçoğlu et al, 2007; Bölüktepe & Yılmaz, 2008).

Honey has high nutritional value, pleasant aroma and taste, and medicinal properties. Its natural nutrients are produced by Apis mellifera species, and they are very useful for human nutrition.

Honey is aromatic and viscous and can be used as a sweetener without any treatment (Haroun, 2006). It is also used as an ingredient in the production of various foods. Therefore, it has a wide range of applications in the food industry and is of great economic importance worldwide (Güler, 2005).

The nutritional value of honey is high; the energy content per 100 grams of honey is 303 (1,379) kcal (kj). Due to the rapid absorption of carbohydrates, honey is also suitable for people of all ages. It is especially recommended not only for children but also patients and elderly people due to its healing effects (Blasa et al, 2006).

Honey quality is evaluated based on the herbal source and chemical composition. Plant source is the most important quality parameter of honey, and the price of honey is often directly related to the herbal source of the honey. The composition of different varieties of herbal honey differs due to the origin of regions in which they are produced (Artık & Konar, 2018).

Honey is composed of 70-80% carbohydrates, 18-20% water, and 1-2% proteins, organic acids, phenolic compounds, and mineral substances. The majority of the carbohydrates in the composition of honey are fructose and glucose, while the rest includes mono-, di- and oligo-saccharides (Saxena et al, 2010). The fructose to glucose ratio is 1.0-1.4 in Turkish flower honey (Artık et al, 2011).

Honey can be classified into two groups based on its source as flower and secretion. Flower honey is produced from nectar collected by honey bees from various plants; e.g., clover, sunflower, acacia, vetch, cotton, and citrus. Secretion honey is produced by the bees from the secretions left by the insects called Basra (Marchelina hellenica) lives on the plants. This instect lives on the plants and produces honey from (Ölmez, 2009). The examples of secretion honeys are pine, fır, and oak (Doğan, 2014).

The content of honey directly depends on the variety and flora (Haroun, 2006). Aroma substances play an important role in determining consumer taste and preference in food. The aroma of various substances is an important criterion that determines the sensory properties of food. Honey has a rich profile of aroma components and contains many volatile compounds (Uçkun, 2011).

The most important part of the aroma in honey is constituted by esters, aldehydes, ketones, alcohols, and volatile acids. Among these compounds, alcohols have the most important place. The aroma substances of honey primarily come from the nectar, which is why honey is named after the aroma of the origin of the nectar as pine honey, etc. (Hışıl & Börekçioğlu, 1986).

Darcy et al, (1997), investigated the volatile components of Australian honey by solvent extraction. The compounds in the extracts were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). As a result of these processes, 55 compounds containing norisoprenoids, monoterpenes, benzene derivatives, aliphatic compounds, and Maillard reaction products were identified. In addition, the following 13 compounds were identified in honey for the first time: four isomeric 3,4-dihydro-3-oxoactinidol, 8,9-dehydrotheaspiron, two isomeric 3-oxoretro-α-ionol, megastigm-4-ene-3,9-dione, 1-phenylbutane-2,3-diol, 1-phenylbutane-2,3-dione, 18-hydroxy oleic acid lactone, 3,5-dihydroxy-2-methyl-4H-pyran-4-one, and 2,5-dimethyl-2,4-dihydroxy-3 (2H) furanone.

In a study conducted by Sunay & Boyacıoğlu (2006), the sensory properties of Turkish honey varieties named according to the region or plant origin were determined by the quantitative descriptive analysis technique. The authors examined 24 different honey samples and determined that especially pine and flower honey had very different descriptive sensory qualities and sensory profiles could be used in both monofloral and polifloral honey.

2. Material and Method

2.1. Source of Honey Samples

Honey samples were obtained from Ordu, Tunceli, İstanbul, Bitlis and Hakkari provinces in Turkey. Each sample was analyzed twice, and the method was proven by the repeatability test.

2.2. Instrumentation

Aroma analysis was performed using Shimadzu QP-2010 model GC-MS equipped with a Restek RTX-5MS (30 m x 0.25 mm x 0.25 30 m) column. A SPME microextraction syringe with a 65 µm PDMS/DVB (Supelco, Bellefonte, PA, USA) (Sánchez-Palomo et al, (2005) fiber was used for collecting the volatile compounds from the headspace of the honey samples.

2.3. Sample Preparations

Many trials were performed with GC-MS in pre-trials. For this purpose, different fibers were tested at different temperatures and times. The GC-MS conditions were changed primarily to pressure and flow. The procedure followed is detailed below.

2 g of each honey sample was weighed in vials.

- The honey samples were heated at 60 °C for 10 minutes without fiber to decompose the aroma substances.
- The honey samples were heated at 60 °C for 40 minutes with fiberglass.
- Fiber was finally injected into GCMS.
- The fiber was conditioned in GC-MS for 10 minutes at 250 °C prior to each injection to eliminate any residues in the fiber from the previous honey sample.

2.4. Determination of the Distribution of Volatile Aromatic Components

The method proposed by Selli et al, (2004) was modified by GC-MS. In the preliminary trials, the temperature and time programs were modified due to the observation of the penetration of the peaks within the first 10 minutes of the injection. After the modification, the peaks were completely separated from each other, and more accurate results were obtained. The must was heated to 40 °C and kept at this temperature for 40 minutes using a 65 µm PDMS/DVB (Supelco, Bellefonte, PA, USA) (Sánchez-Palomo et al, 2005) fiber. The fiber was then injected into GC-MS to perform the analysis. The GC-MS parameters of the method used in our research were as follows: injection temperature 250 °C, pressure 49.7 kPa, column flow rate 1.00 mL/min 2, 240 °C, waiting time at last temperature 10 min, and split ratio 1/10. In order to accurately interpret the peaks obtained after injection, the method parameters of the C7-C30 alkane series were injected into the device and three different (Wiley, FFNSC and NIST) GC-MS libraries were identified. The volatile aroma components found in the must were determined based on a similarity of 85% over the carbon series, and the data were determined as the percentage of the areas of the identified peaks in the total area.

2.5. Source of Honey Samples

Solid phase microextraction (SPME) is a simple and solvent-free sample preparation technique based on the adsorption of volatile components onto the fiber and the desorption of these components from the fiber by GC at the injection port.

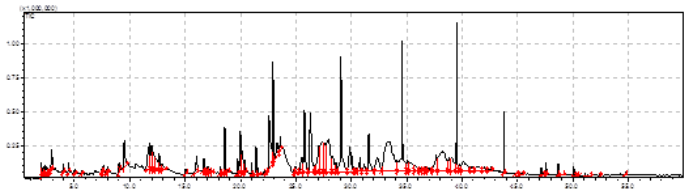
2.6. GC-MS.

2.6.1. Fiber conditioning

In each new sample, the fiber was conditioned for 10 minutes at 250 ºC, and a fiber tip was replaced in five samples.

2.6.2. Fiber conditioning

Unknown compounds were identified taking into account the MS spectra of volatile compounds separated by retention times and NIST/EPA/NIH Mass spectral library (NIST 98) compared to authentic standards (Odeh et al, 2007). The GCMS chromatogram of the honey sample collected from Ordu province is shown in Figure 1.



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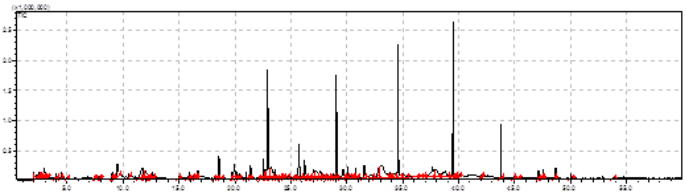

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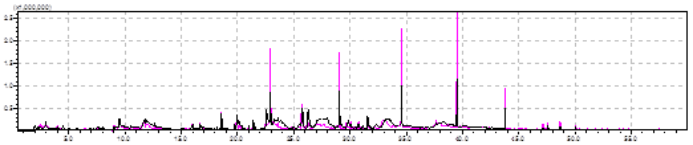

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3. Results and Discussion

In this study, the honey samples collected from five different regions of Turkey were analyzed. Since honey is a very complex mixture, aroma substances varied in the samples collected from different regions. The most volatile aroma compounds were found in Bitlis honey, and the least in Hakkari honey. The common components found in most honey varieties are given in Table 1. Acetic acid, furfural, benzaldehyde, benzeneacetaldehyde, benzoic acid and hexanoic acid were found in all five honey samples..

The defined compounds can be divided into different chemical classes: alcohols (e.g. ethanol and 1-butanol), phenols (e.g., phenol and 2-methoxy-4-vinylphenol), ketones (e.g., acetone), organic acids (e.g., acetic and butanoic acid), esters (e.g., ethyl acetate), aldehydes (e.g. furfural and nonanal), aliphatic hydrocarbons (e.g., octane and nonane), aromatic hydrocarbons (e.g., toluene), amino acids (dl-alanine), and benzyl derivatives (benzyl alcohol and benzoic acid) (Wolski et al, 2006).

Alcohols are formed as a result of the oxidative degradation of lipids in honey or the catalyzing aldehydes of reductase enzymes contaminated with honey (Moreira et al, 2010).

Soria et al, (2003), evaluating the phenol compound of the aroma substances in honey, reported the amount of this compound as 4.3 µg/kg. In a study on chestnut and lemon honey, Guyot et al, (1998) detected the phenol compound.

Piasenzotto et al, (2003), investigated the Italian flower honey and detected that furfural, phenyl acetaldehyde, 3-4-5 trimethoxy benzaldehyde compounds as a flavoring agent of the aldehyde group.

Castro-Vázquez et al, (2003) stated that furfural gave honey almond and bread odor, benzaldehyde resulted in almond odor, and phenylacetaldehyde was responsible for the honey-like odor.

Alissandrakis et al, (2009) identified volatile compounds of citrus, chestnut, thyme, pine, acacia, highland and cotton honey samples and found that the compounds obtained mostly belonged to hydrocarbon, alcohol, phenol, aldehyde and ketone groups.

Piasenzotto et al, (2003), analyzed 40 honey samples obtained from different regions of Italy, including five citrus, 10 chestnut, eight eucalyptus, 11 lime, two thyme and four dandelion honey samples and reported that the compounds of the same origin exhibited a similar profile, while some components were only detected in a particular honey sample.

In this study, the components identified in different honey varieties were limonene diol in citrus honey; trans-rose oxide, cis-Rose oxide, p-methyl acetophenone, carvacrol and 8-p-menthen-1,2-diol in lime honey; nitrile compounds in dandelion honey; acetophenone and 1-phenylethanol components in chestnut honey; and acetoin in eucalyptus honey. 2-Phenylethanol was detected in the honey samples of all different plant origins.

In their study that aimed to determine the botanical origin of honey based on volatile aroma substances, Cuevas-Glory et al, (2007), successfully distinguished between eucalyptus and citrus honey using the aroma substances they contained. The authors stated that methyl anthranilate, lilac aldehyde, limonene diol, hotrienol and 1-p-menthen-al compounds characterized citrus honey while nonanol, nonanal, nonanoic acid, and acetoin characterized eucalyptus honey.

Alissandrakis et al, (2009) found the compounds of phenyl ethyl alcohol and 3-methyl-1-butanol in their study on Greek thyme honey. Phenyl ethyl alcohol has also been previously reported to be a compound responsible for rose and floral odors in honey (Etschmann et al, 2002).

Castro-Vázquez et al, (2003) reported 3-methyl-1-butanol compounds as 36.2 µg/kg in seven flower honey. Pino, (2012) reported that the 3-methyl-1-butanol compound provided the almond and fruity odor.

In the current study, the honey variety from Ordu had the highest amount of three components, namely triacetin (10.82 %), benzoic acid (8.94%) and benzeneacetaldehyde (6.47%). These components characterized the quality of Ordu honey. Benzoic acid gives an aroma of fruity grapes.

Honey collected from Hakkari attracted attention with a high rate of (47.8%) hotrienol, which has a moldy aroma. The other two components in this honey were furfural at 6.99% and benzeneacetaldehyde at 7.04%. Benzeneacetaldehyde has a sweet, refreshing aroma similar to peppermint while furfural has a spicy aroma.

In Bitlis honey, there were three components with the highest amountsÇ acetic acid (8.86%), pelargonaldehyde (7.44%), and caprylic acid (7.09%). While acetic acid gives a musky or coumarin-like aroma, pelargonaldehyde provides a fresh, fruity aroma.

Among the honey samples examined, the furfural content of Çanakkale honey (25.13%) was the highest. Furfural is a good quality marker in honey. An excessive amount of furfural may indicate loss of freshness of honey or exposure of honey to high temperatures (Castro-Vazquez et al, 2003). The other two high-level components of this honey were hexane (8.76 %) and methyl 2-furoate (6.88 %).

For Tunceli honey, furfural (12.37%) was found to be at the highest proportion, followed by acetic acid and hexanoic acid at the same percentages (7.94%). In Tunceli honey, the furfural ratio was as high as in Çanakkale honey.

Table 1. Volatile compounds characterized by GC–MS in five samples of Turkish honey

Peak	Compounds	Min %	Max %	Mean concentration %
1	Dl-Alanine	1.31	5.89	2.78
2	Acetic acid	4	8.86	5.85
3	Butane	1.07	5.96	2.94
4	Furfural	4.43	25.13	12.37
5	Benzaldehyde	0.94	2.08	1.57
6	Benzeneacetaldehyde	2.79	7.04	4.96
7	Benzoic Acid	0.79	8.94	2.60
8	Benzoic acid, 2-ethylhexyl ester	0.76	1.70	0.96
9	Ethanol	1.07	6.22	4.22
10	Nonanal	1.13	4.06	1.41
11	Nonanoic acid	0.58	3.92	2.34
12	Hexanoic acid	2.57	7.94	6.47
13	Octanoic Acid	2.12	5.72	4.68
14	Methyl 2-furoate	3.35	6.88	3.85
15	Ethylhexanoic acid	1.88	4.53	3.27

4. Conclusions

The origin and flora of honey are of great importance in the Turkish and global markets, but consumers are misled by imitations and adulteration. Since there is no geographical registration in the honey sold, some consumers are deceived into buying low-quality honey at high prices. For this reason, more studies on local or monofloral honey can be performed and geographic registration can be made by determining the characteristics and marker components of honey. Registered products will have a chance to be marketed as high value added products in both domestic and foreign markets. Thus, not only will honey producers gain but this will also contribute to the national economy and ensure consumer access to reliable food.

This study showed that the analysis of aroma components with GCMS was a very effective method for determining the botanical origin of honey.

Some of the components were found in only certain samples. Thus, identifying these specific components is important in determining the unique character of each honey variety that differentiates it from different products. For example, in this study, the compounds of dimethyl ether, acetone, lactic acid, senecioic acid, linalool oxide, benzyl carbinol, triacetin, butyl butyrate, 1-hexadecanol, propanoic acid, 2-oxo-, furfuryl alcohol, isobutyl acetate, 1,3-dioxolane-4-methanol, and decanal were obtained only from Ordu honey.

In addition, some components were present in all honeys, but their proportions were different. Acetic acid, furfural, benzaldehyde, benzeneacetaldehyde, benzoic acid, and hexanoic acid were found in all five honey samples examined. The differences in the proportions of these components determined the character of honey.

One of the greatest possibilities for an analyst is the GC-MS combination. The chromatographic column provided the conditions that well distinguish tens or even hundreds of components in its matrix. These components are single mass spectrometer device. The spectra showing the particles are examined to elucidate the structure of the compound using the spectral library of the device.

The determination of the aromatic components of honey by the SPME-GC/MS technique can lead to bright ideas for the future. With this technique, the botanical identity and geographical origin of honey can be easily determined in the future. For this purpose, it is necessary to increase the number of studies on honey from various botanical sources (Wolski et al, 2006).

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