The Microscopic and GC-MS Analysis of Turkish Honeydew (Pine) Honey

Türk Çam Ballarının Mikroskobik ve GC-MS Analizi

Research Article

Aslı Özkök[1], Kadriye Sorkun[2], Bekir Salih[3]

[1]Hacettepe University, Faculty of Science, Bee and Bee Products Research and Application Center, Beytepe, Ankara, Turkey.
[2]Hacettepe University, Faculty of Science, Biology Department, Beytepe, Ankara, Turkey.
[3]Hacettepe University, Faculty of Science, Chemistry Department, Beytepe, Ankara, Turkey.

ABSTRACT

78 honey samples were collected from September to November between the years 2004-2006 where the most of the honeydew beekeeping process is realized in Muğla city in Turkey. All honey samples were investigated with microscope and were checked the Number of Honeydew Elements (NHE) and the Number of Total Pollen (NTP). The quality of honey samples was determined by correlating NHE to NTP ratio and the honey which has NHE to NTP ratio bigger than 4.5 was accepted as a high density superior quality honeydew (pine) honey. As a result of identifications which have been made in microscope, 50 honey samples of 78 honey samples were selected as high quality pine honey and they were appropriate ones to study for GC-MS chemical compounds analysis. As a result of GC-MS chemical compounds analysis of 50 honeydew honeys were found chemical compounds which are important for human body. These are aromatic aldehydes, aldehydes, aromatic alcohols, flavanones, linear hydrocarbons, aromatic hydrocarbons, aromatic acids, aromatic acid esters, linear acid esters and other chemical compounds.

Key Words
Honeydew (pine) honey, *Marchallina hellenica*, chemical compounds analysis with GC-MS.

ÖZ

Türkiye'de arıcılığın yoğun olarak yapıldığı Muğla ilinden 2004-2006 yılları arasında Eylül ayından Kasım ayına kadar 78 çam balı örneği toplanmıştır. Toplanan bal örneklerinde mikroskop ile Balçiği Element Sayısına (NHE) ve Toplam Polen Sayısına (NTP) bakılmıştır. Bal örneklerinin kalitesi Balçiği Elementlerinin Sayısının Toplam Polen Sayısına oranı ile belirlenmektedir. Balçiği Elementi Sayısının Toplam Polen Sayısına oranı 4.5'un üstündeki ballar çok yoğun üstün kaliteli çam balı olarak kabul edilmektedir. Mikroskopta yapılan analiz sonucunda 78 bal örneğinin 50 tanesi çok yoğun üstün kaliteli çam balı olarak bulunmuştur. Ayrıca GC-MS analizlerinde balın kimyasal bileşenlerine bakılmıştır. 50 çam balı örneğinin GC-MS kimyasal bileşen analizinde insan sağlığı için önemli bileşenler bulunmuştur. Bunlar aromatik aldehitler, aldehitler, aromatik alkoller, flavanonlar, lineer hidrokarbonlar, aromatik hidrokarbonlar, aromatik asitler, aromatik asit esterleri, lineer asit esterleri ve diğer kimyasal bileşenlerdir.

Anahtar Kelimeler
Çam balı, *Marchallina hellenica*, GC-MS ile kimyasal bileşen analizi.

Article History: Received: Mar 02, 2016; Revised: Sep 04, 2016; Accepted: Oct 16, 2016; Available Online: Dec 31, 2016.

DOI: 10.15671/HJBC.2016.117

Correspondence txxo: A. Özkök, Hacettepe University, Faculty of Science, Bee and Bee Products Research and Application Center, Ankara, Turkey.
Tel: +90 312 780 7161 Fax: +0312 299 2028 E-Mail: aozkok@hacettepe.edu.tr

INTRODUCTION

Honeydew honey is prepared from secretions of living parts of plants or excretions of plant-sucking insects on the living part of plants [1].

Honeydew is the origin of pine honey, which is a classification of honey. It refers to honey produced by honeybees collecting nectars which are exuded from other insects such as aphids or scale insects [2]. Insects take essential nutrition from concentrated sugar solution in the floem and exude the remains. Honeybees take these remains and bring to the hive and turn into honey. This honey is called honeydew honey [2].

Honeydew honey is generally characterized by honeydew elements composed of microscopic algae, fungus spores. If a honey with the ratio "number of honeydew elements"/"number of total pollens" is greater than 3, is considered as honeydew honey [3,4].

In Turkey *Marchalina hellenica* (syn. *Monophlebus hellenicus*) (Coccidea: Homoptera), which lives on *Pinus brutia*, is the main source of honeydew. The habitat of this insect is only Turkey and Greece [5]. *Marchalina hellenica* mainly is found in Southern Marmara, the Aegean and West Mediterranean regions of Turkey [6]. Muğla is one of the best places for pine honey which has been produced by *Marchalina hellenica*. In Turkey, about 30% of honey is produced in the region of Muğla. Muğla, having nearly 60 000 hectare of *Pinus brutia* forest, is a very important city for the production of pine honey [7].

MATERIALS and METHODS

Collection of Honey Samples

78 honey samples were collected from ten areas of the Muğla city in western Turkey, Merkez, Milas, Ortaca, Köyceğiz, Marmaris, Fethiye, Yatağan, Bodrum, Ula and Datça where pine honey beekeeping is practiced extensively, from September to November between the years 2004-2006. In this study, suitable apiaries were chosen from villages. It was important that the villages are separated with enough distance and there are enough vegetation differences, which also represent Muğla statistically.

Microscopic Analysis of Honeys

Preparates to identify NTP and NHE in 10 grams of honey are obtained as follows [8,9]: 500 grams of stock honey was well stirred with a sterile glass stick and 10 grams of it was separated. Then 20 ml distilled water was added and the mixture was placed in a tube together with a tablet as witness containing 12542 *Lycopodium* spores. To melt down the tablet, tubes were left for 10-15 minutes in a water bath of 45°C. After the tablet fully melted, few drops of basic fuchsine were added for colouring pollens and spores and then the material was centrifuged in 3500 for 45 minutes. Water in centrifuged tubes was removed and tubes were left upside down on a drying mat for full drainage. Homogenous mixing was ensured by adding 1 ml of 50% glycerine to each tube. 0.01 ml was taken from this mixture and plated on a lam. The material was covered by a lamella of size 18x18 mm^2 and two separate preparates were obtained for microscopic analysis.

Examination of the Number of Total Pollens (NTP)

Pollen and spore preparates were examined and counted under a Nicon Eclipse E400 light microscope. Objectives of 20x and 40x were used in counting pollens. During the counting process, the preparate was examined starting from the top left corner and by fully scanning the area of size 18x18 mm^2 the numbers of all pollens and *Lycopodium* spores in this area were taken separately. Counts of two separate preparates were taken and their averages were applied to the formula given below. The resulting figure is the total number of pollens in 10 grams of honey.

Number of Total Pollens (NTP)/10 gram = [Pollens counted x 12542*] / *Lycopodium* Spores counted

*Number of spores found in a *Lycopodium* tablet

Examination of the Number of Honeydew Elements (NHE)

In the same preparates in which NTP was counted, the number of honeydew elements (NHE) was also counted. During this process again, starting from the top left corner and by fully scanning the area of size 18x18 mm^2 the numbers of all spores, hyphea and, if there is any, algae were taken. The

NHE content in 10 grams of honey was found by using the following formula:

The Number of Honeydew Elements (NHE)/10 g = [Number (spore + hyphea + algae) counted] / x 12542 *Lycopodium* Spores counted

NHE/NTP ratio

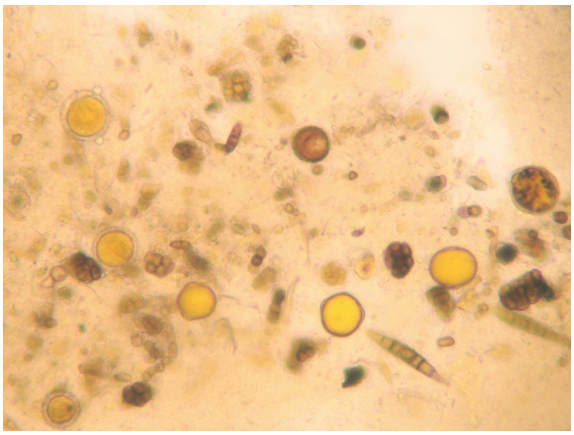
According to results obtained by using NHE/NTP ratio a pine honey can be classified as High Density-Superior Quality Pine Honey, Dense Pine Honey, Floral Honey Added Pine Honey and Low Density Floral Honey [3,7,10]. The table below gives honey types and classes on the basis of NHE/NTP ratio (Table 1).

Table 1. Classification of honey samples by NHE/NTP ratio.

NHE/NTP	Identification	Honey type
0-1.5	Low density	Floral honey
1.5-3.0	Medium density	Pine + floral honey
3.0-4.5	Dense	Pine honey
> 4.5	High dense	Superior quality pine honey

Figure 1 is the microscopic view of High Dense-Superior Quality Pine Honey. The picture exhibits the concentration of honeydew elements (spores and hyphea) and few pollens.

Figure 1. Microscopic view of high density-superior quality pine honey.



Following microscopic examinations, 50 honey samples were determined as high density-superior quality pine honey samples and found to be appropriate for GC-MS chemical compounds analysis.

Pine Honey Samples and Preparation of Extracts for the Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS was used for chemical compounds analysis of the pine honeys. The Method for GC-MS was performed in accordance with Barcarola et al, [11]; Radovic et al., [12]; Soria et al., [13]; Cuevas-Glory et al., [14]. According to these methods 10 gr. pine honey dissolved in 10 ml methanol and was evaporated to dryness. After drying, 10 ml ethanol was added to the tubes. This extraction was left for 24 hours as the mouth is closed. This solution is evacuated to another tubes at the end of 24 hours and is waited alcohol for flying. After flying completely alcohol 0.5 ml ethanol was added to the residue in the bottom of the beaker and thoroughly mixed a thin pipette. 2 μl GC-MS injection was performed.

GC-MS Analysis of Pine Honey Samples

A GC 6890N from Hewlett-Packard (Palo Alto, CA, USA) coupled with mass detector (MS5973, Hewlett-Packard) was used for the analysis of EEP samples. Experimental conditions of GC-MS system were as follows: DB 5MS column (30 m×0.25 mm and 0.25 μm of film thickness) was used and flow rate of mobile phase (He) was set at 0.7 ml/min. In the gas chromatography part, temperature was kept at 50°C for 1 min. and then increased to 150°C with 10°C/min heating ramp. After this period, temperature was kept at 150°C for 2 minutes. Finally, temperature was increased to 280 with 20°C/min heating ramp and then kept at 280°C for 49 minutes and chemical substances of the pine honey samples were identified by using standard Willey and Nist Libraries available in the data acquisition system of GC–MS if the comparison scores were obtained higher than 90%.

RESULTS

Table 2 shows the microscopic analysis of 78 honey samples. As a result of identifications which have been made in microscope, 50 honey samples were selected as high density superior quality pine honey and they were appropriate

Table 2. Outcomes of NHE/NTP analysis of honey samples.

No	District-village	NHE	NTP	NHE/NTP	Identification
1	Yatağan-Şerefköy	2528	93919	0.02	Low density floral honey
2	Yatağan-Şerefköy	2752	6882	0.4	Low density floral honey
3	Yatağan-Hacıveliler	3836	16526	0.23	Low density floral honey
4	Yatağan-Hacıveliler	15176	58178	0.26	Low density floral honey
5	Yatağan-Turgutlar	2068	50685	0.04	Low density floral honey
6	Yatağan-Turgutlar	6318	225947	0.03	Low density floral honey
7	Ula-Kıyra	31412	17816	1.76	Medium density pine + floral honey
8	Ula-Kıyra	58946	16806	3.50	Dense pine honey
9	Datça-Palamutbükü	26684	15121	1.76	Medium density pine + floral honey
10	Datça-Palamutbükü	12656	128411	0.09	Low density floral honey
11 (1)	**Datça-Sındı**	**59408**	**9995**	**5.94**	**High density superior quality pine honey**
12 (2)	**Datça-Sındı**	**55770**	**10940**	**5.09**	**High density superior quality pine honey**
13 (3)	**Datça-Hızırşah**	**43326**	**8795**	**4.92**	**High density superior quality pine honey**
14	Datça-Hızırşah	31418	13175	2.38	Medium density pine + floral honey
15 (4)	**Marmaris-Çamlı**	**166366**	**9222**	**18.04**	**High density superior quality pine honey**
16 (5)	**Marmaris-Çamlı**	**189892**	**11758**	**16.15**	**High density superior quality pine honey**
17	Marmaris-Çamlı	127180	39606	3.21	Dense pine honey
18	Marmaris-Çamlı	79812	38462	2.07	Medium density pine + floral honey
No	**District-village**	**NHE**	**NTP**	**NHE/NTP**	**Identification**
19 (6)	**Marmaris-Çetibeli**	**66284**	**12736**	**5.2**	**High density superior quality pine honey**
20	Marmaris-Çetibeli	58290	1247272	0.04	Low density floral honey
21 (7)	**Marmaris-Turgut**	**118880**	**26391**	**4.5**	**High density superior quality pine honey**
22	Marmaris-Turgut	87082	20734	4.2	Dense pine honey
23 (8)	**Marmaris-Orhaniye**	**168200**	**28623**	**5.87**	**High density superior quality pine honey**
24 (9)	**Marmaris-Orhaniye**	**128168**	**12370**	**10.36**	**High density superior quality pine honey**
25 (10)	**Ortaca-Karadonlar**	**55944**	**1832**	**30.5**	**High density superior quality pine honey**
26	Fethiye-Çatak	3754	6608	0.56	Low density floral honey
27	Fethiye-Çatak	8946	6390	1.4	Low density floral honey
28 (11)	**Fethiye-Dere**	**498544**	**4703**	**106**	**High density superior quality pine honey**
29 (12)	**Fethiye-Dere**	**146542**	**8449**	**17.3**	**High density superior quality pine honey**
30	Fethiye-Bağlıağaç	40980	42730	0.95	Low density floral honey
31	Fethiye-Bağlıağaç	24454	12069	2.02	Medium density pine + floral honey
32	Fethiye-Kabaağaç	3622	28428	0.12	Low density floral honey
33	Fethiye-Kabaağaç	13682	23183	0.59	Low density floral honey
34 (13)	**Centre-Dağpınar**	**115481**	**1419**	**81**	**High density superior quality pine honey**
35 (14)	**Centre-Dağpınar**	**65323**	**871**	**75**	**High density superior quality pine honey**

Table 2. Outcomes of NHE/NTP analysis of honey samples. (continue)

36 (15)	**Centre-Kıran**	**138817**	**6271**	**22.13**	**High density superior quality pine honey**
37 (16)	**Centre-Kıran**	**136963**	**13318**	**10.28**	**High density superior quality pine honey**
38 (17)	**Ula-Akyaka**	**70007**	**14366**	**4.87**	**High density superior quality pine honey**
39 (18)	**Ula-Akyaka**	**184709**	**3990**	**46.3**	**High density superior quality pine honey**
40 (19)	**Ula-Akçapınar**	**137840**	**11933**	**11.5**	**High density superior quality pine honey**
41 (20)	**Ula-Akçapınar**	**102744**	**1605**	**64**	**High density superior quality pine honey**
42 (21)	**Datça-Sındı**	**65497**	**1950**	**33.5**	**High density superior quality pine honey**
43 (22)	**Datça-Sındı**	**66041**	**10778**	**6.12**	**High density superior quality pine honey**
44 (23)	**Marmaris-Osmaniye**	**127564**	**4502**	**28**	**High density superior quality pine honey**
45 (24)	**Marmaris-Osmaniye**	**106746**	**5016**	**21**	**High density superior quality pine honey**
46	Fethiye-Ören	22717	7809	2.9	Medium density pine + floral honey
47 (25)	**Fethiye-Ören**	**89884**	**10451**	**8.6**	**High density superior quality pine honey**
48 (26)	**Fethiye-Ören**	**116258**	**6107**	**19**	**High density superior quality pine honey**
49 (27)	**Fethiye-Ören**	**116040**	**5889**	**19**	**High density superior quality pine honey**
50 (28)	**Ortaca-Gökbel**	**123341**	**12888**	**9.5**	**High density superior quality pine honey**
51 (29)	**Ortaca-Gökbel**	**121619**	**5320**	**22.8**	**High density superior quality pine honey**
52	Köyceğiz-Döğüşbelen	95401	113494	0.84	Low density floral honey
53 (30)	**Köyceğiz-Döğüşbelen**	**118916**	**19277**	**6.16**	**High density superior quality pine honey**
54	Köyceğiz-Döğüşbelen	82534	25893	3.1	Dense pine honey
55	Köyceğiz-Döğüşbelen	70742	25647	2.7	Medium density pine + floral honey
56 (31)	**Milas-Bozbük**	**75542**	**16802**	**4.5**	**High density superior quality pine honey**
57 (32)	**Milas-Bozbük**	**142085**	**11854**	**11.9**	**High density superior quality pine honey**
58 (33)	**Milas-Pınar**	**213810**	**2388**	**89.53**	**High density superior quality pine honey**
59 (34)	**Milas-Pinar**	**67215**	**731**	**92**	**High density superior quality pine honey**
60 (35)	**Milas-Kayabükü**	**110731**	**7231**	**15.3**	**High density superior quality pine honey**
61 (36)	**Milas-Kayabükü**	**135453**	**5495**	**24.6**	**High density superior quality pine honey**
62 (37)	**Yatağan-Bencik**	**152800**	**3200**	**47**	**High density superior quality pine honey**
63 (38)	**Yatağan-Bencik**	**223367**	**3400**	**77**	**High density superior quality pine honey**
64 (39)	**Yatağan-Bencik**	**121500**	**4507**	**26.9**	**High density superior quality pine honey**
65 (40)	**Yatağan-Bencik**	**143606**	**2508**	**57.2**	**High density superior quality pine honey**

Table 2. Outcomes of NHE/NTP analysis of honey samples. (continue)

66 (41)	**Milas-Çukurköy**	**214864**	**9241**	**23.2**	**High density superior quality pine honey**
67 (42)	**Milas-Çukurköy**	**167851**	**5860**	**28.6**	**High density superior quality pine honey**
68 (43)	**Ula-Karaböğütlen**	**51719**	**7499**	**6.9**	**High density superior quality pine honey**
69 (44)	**Ula-Karaböğütlen**	**46747**	**7069**	**6.6**	**High density superior quality pine honey**
70 (45)	**Köyceğiz-Toparlar**	**61548**	**4645**	**13.25**	**High density superior quality pine honey**
71	Köyceğiz-Toparlar	26977	15224	1.7	Medium density pine + floral honey
72 (46)	**Datça-Mesudiye**	**77949**	**5124**	**15.2**	**High density superior quality pine honey**
73 (47)	**Datça-Mesudiye**	**92446**	**9507**	**9.7**	**High density superior quality pine honey**
74	Yatağan-Bağyaka	266578	232395	1.14	Low density floral honey
75	Ula-Kızılyaka	182940	224242	0.81	Low density floral honey
76 (48)	**Bodrum-Gölköy**	**316190**	**40926**	**7.72**	**High density superior quality pine honey**
77 (49)	**Köyceğiz-Ekincik**	**390004**	**32987**	**11.82**	**High density superior quality pine honey**
78 (50)	**Fethiye-Kabaağaç**	**192481**	**23292**	**8.26**	**High density superior quality pine honey**

*Bold coloured samples were chosen for the GC-MS analysis. Sample numbers were written between brackets.

Table 3. Chemical compositions pine honey samples (% content).

	Aromatic aldehydes	Aldehydes	Aromatic Alcohols	Flavanones	Linear Hydrocarbons		Aromatic Hydrocarbons		Aromatic acids	Aromatic acid esters	Linear fatty acid esters	Other Chemicals
Sample no	Furfural	2-Furancarboxaldehyde	2-Furanmethanol	4H-Pyran-4-one, 2,3-dihydro-3,5-dihyroxy-6-methyl	Eicosane	Heptacosane	Benzene	Octadecane	Benzoic acid	1,2-Benzenedicarboxylic acid	Octadecenoic acid methyl ester	Lidocaine
1	-	-	-	-	1.02	-	28.94	-		-	1.78	5.64
2	-	-	-	-	-	-	-	-	-	-	-	3.74
3	-	-	0.86	-	0.39	0.65	-	-	-	-	-	2.52
4	-	-	0.96	1.38	0.33	-	-	0.44	-	-	-	2.41
5	-	-	-	-	-	-	27.67	-	-	-	-	1.02
6	-	-	1.91	2.33	0.11	0.14	-	-	-	-	-	3.21
7	-	-	-	-	-	-	-	-	-	-	-	3.59
8	-	-	1.14	1.50	-	-	-	-	-	-	-	5.19
9	0.35	-	1.30	2.43	0.29	0.36	-	-	2.28	-	-	3.90
10	-	-	-	-	1.79	1.89	-	-	1.86	-	-	7.53
11	-	-	1.92	-	-	-	-	-	-	-	-	0.95
12	-	-	-	-	0.46	-	-	-	-	1.81	-	2.01
13	-	16.27	2.08	1.56	-	-	8.07	-	-	-	-	1.21
14	-	-	1.15	-	1.24	-	8.16	-	-	-	-	2.93
15	-	-	1.78	-	0.98	1.41	-	-	-	-	-	1.81

Table 3. Chemical compositions pine honey samples (% content). (continue)

16	0.47	7.51	0.94	-	0.36	-	-	-	-	-	0.33	3.86
17	-	-	1.06	1.59	1.03	-	-	-	0.69	-	-	2.74
18	0.81	-	0.90	-	0.67	0.51	1.03	-	0.98	-	0.62	2.76
19	0.56	-	1.37	-	-	0.55	-	-	0.65	-	0.98	4.88
20	-	-	1.28	1.74	0.58	-	-	-	1.31	-	-	4.50
21	-	-	1.47	2.55	1.57	-	-	-	-	-	-	4.05
22	-	-	1.46	2.41	1.44	-	-	-	-	-	-	2.43
23	0.25	-	1.42	-	-	-	-	-	-	-	-	0.83
24	-	-	1.48	2.38	0.96	1.37	-	-	-	-	-	2.86
25	-	-	-	-	-	-	0.75	-	-	-	-	0.98
26	-	-	1.08	1.64	-	-	-	-	-	-	-	3.52
27	-	-	1.33	2.77	0.38	-	4.86	-	-	-	0.44	2.50
28	-	-	1.40	2.47	1.07	-	-	-	1.47	-	-	8.86
29	0.51	5.80	1.78	2.01	-	-	-	-	-	-	-	2.91
30	-	-	1.38	2.36	2.74	-	-	-	0.63	-	-	3.36
31	-	-	0.93	-	3.3	-	-	-	-	-	-	2.82
32	-	-	-	-	0.49	-	-	-	-	-	-	3.68
33	0.49	-	1.10	2.14	-	-	-	-	-	-	-	2.27
34	-	-	1.20	2.01	-	-	-	-	0.64	-	-	3.10
35	-	13.05	1.29	3.31	1.43	-	-	-	-	-	0.36	1.55
36	-	-	1.57	-	0.70	0.94	-	-	-	-	-	2.04
37	0.73	-	1.80	-	0.44	-	-	-	-	-	0.12	2.63
38	-	-	1.49	-	1.44	-	3.19	-	0.76	-	-	2.35
39	-	-	1.05	2.07	0.66	0.91	-	-	-	-	0.44	2.89
40	-	-	0.87	-	3.23	-	1.73	-	-	-	0.57	2.12
41	-	-	1.02	-	1.36	-	-	-	-	-	-	2.19
42	-	-	1.15	-	0.37	1.30	13.53	-	-	-	-	1.78
43	-	-	1.35	2.37	0.77	-	-	-	-	-	-	4.46
44	-	-	-	-	-	-	9.75	-	1.12	-	-	3.35
45	-	-	1.36	2.82	-	-	-	-	-	-	-	2.58
46	-	-	1.64	2.57	-	1.19	-	-	1.26	-	-	4.67
47	-	-	1.35	2.26	-	-	-	-	-	-	0.52	3.46
48	-	-	1.52	2.54	-	-	-	-	-	-	1.13	3.13
49	-	-	1.17	-	1.51	1.36	-	-	-	-	1.01	2.34
50	1.33	-	0.57	1.98	-	-	-	-	-	-	-	2.09

ones to study for GC-MS chemical compounds analysis (Table 3).

DISCUSSION

GC-MS chemical compounds analysis of 50 honeydew honeys revealed aromatic aldehydes, aldehydes, aromatic alcohols, flavanones, linear hydrocarbons, aromatic hydrocarbons, aromatic acids, aromatic acid esters, linear acid esters and other chemical compounds.

One of the aromatic aldehydes furfural was found in nine of the samples which has been determined before in acacia honey by Radovic et al. [12]. Furfural was also determined in lime and lavender honey by Cuevas-Glory et al., [14]. It is toxic substance with an LD_{50} of 65 mg/kg (oral, rat).

2-Furanmethanol also called Furfuryl alcohol was found generally in all pine samples. This substance was previously detected in honey by Radovic et al. [12], Allissandrakis et al. [15]. It finds use as a solvent, but is primarily used as an ingredient in the manufacture of various chemical products such as foundry resins, adhesives, and wetting agents.

Flavanoids are important compounds because of their antioxidant effects. Flavanones are one of the most important groups of the flavonoids. Included in the group of flavanones 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) which is a strong antioxidant [16] was detected in most of the pine honey samples. Especially 21, 27, 35, 45, 46 and 48. samples contained high concentrations of DDMP. Shimoda et al. [17] also determined this substance in honey.

One of the linear hydrocarbons Eicosane and Heptacosane was found generally in all pine samples. Bentivenga et al. [18] has also determined Heptacosane in beeswax and honey.

The presence of aromatic hydrocarbons in honey is an indicator of the environmental problems. This types of components are generally used as indicators of aquatic environmental pollution [18]. Aromatic hydrocarbons were identified in high concentrations, especially 1, 5, 13, 14, 42, 44. samples. These samples can be used as indicators of the dirty regions.

Benzoic acid which is an aromatic acid occurs naturally in many plants. It has been found in twelve of the pine samples. Benzoic acid was used as an expectorant, analgesic, and antiseptic in the early 20th century and it is still used for different purposes.

1,2-Benzenedicarboxylic acid also called Phthalic acid is one of the aromatic acid esters and is produced by the catalytic oxidation of naphthalene. It is a toxic compound and fortunately was found very low concentration only in one sample.

Octadecanoic acid methyl ester also called Stearic acid is one of the linear fatty acid esters and it is nature's most common long-chain fatty acids, derived from animal and vegetable fats. It is widely used as a lubricant and as an additive in industrial preparations. It is used in the manufacture of metallic stearates, pharmaceuticals, soaps, cosmetics, and food packaging. This compound was found in low concentrations in twelve of the samples.

Lidocaine was also detected in all pine honey samples for the first time in our study. Especially 1, 8, 28. samples contained the high concentrations of Lidocaine. Lidocaine is used topically to relieve itching, burning, and pain from skin inflammations, injected as a dental anesthetic, or used as a local anesthetic for minor surgery. This local anesthetic compound was previously found in honey by Yan and Yu-Hong [19]. This may suggest that pine honeys may be more effective in the treatment of wounds and burns.

As a conclusion, this study is important as it determines the chemical characterization profile of pine honeys which are endemic to Turkey and shed light on the standard data. Further chemical investigations are needed for evaluating Turkish pine honeys.

ACKNOWLEDGEMENTS
We are indebted to the The Scientific and Technological

Research Council of Turkey (TÜBİTAK) for financial support, and also to Muğla Beekeepers' Association (MAYBİR) and local people who helped us during our fieldwork.

References

1. M.L. Sanz, M. Gonzalez, C. Lorenzo, J. Sanz, I. Martinez-Castro, A contribution to the differentiation between nectar honey and honeydew honey, Food Chemistry, 91 (2005) 313-317.
2. E. Zander, A. Koch, Der Honig, Eugen Ulmer Verlag, Stuttgart, (1994) 201 p.
3. J. Louveaux, A. Maurizio, G. Vorwohl, International Commission for Bee Botany of IUBS, Methods of Melissopalynology, Bee World, 59 (1978) 139-157.
4. A.C. Soria, M. Gonzalez, C. Lorenzo, I. Martinez-Castro, J. Sanz, Characterization of artisanal honeys from Madrid (Central Spain) on the basis of their melissopalynological, physicochemical and volatile composition data, Food Chemistry, 85 (2004) 121-130.
5. L.A Santas, *Marchalina hellenica* An Important Insect for Apiculture of Greece, The XXVIIth International Congress of Apicultur of Apimondia, Athens, (1979) 419-422.
6. B. Gürkan, Çam pamuklu koşnili *Marchalina hellenica* (Genadius)'nın Biyo-Ekolojisi ve Populasyon Dinamiği. Doktora Tezi, Ankara (1989).
7. A. Şahin, Marmaris-Muğla Yöresinde Üretilen Çam Ballarının Mikroskobik Analizi ve Organoleptik Özelliklerinin Saptanması, Yüksek Lisans Tezi, Ankara (2000).
8. N.T. Moar, Pollen analysis of New Zealand honey, New Zealand Journal of Agricultural Research, 28 (1985) 38-70.
9. K. Sorkun, Türkiye'nin Nektarlı Bitkileri, Polenleri ve Balları, Palme Yayıncılık, (2008) 341s.
10. R. Sawyer, Honey Identification, Cardiff Academic Press, UK, (1988) 109 p.
11. R. Barcarola, M. Centeleghe, P. Zanatta, L.S. Cont, GC-MS coupled with headspace sampling with reverse carrier flow in sampling step applied to honey characterization, In: 5th international symposium of hyphenated technique in chromatography. Bruges, Belgium (1998) 11-21 p.
12. B.S. Radovic, M. Careri, A. Mangia, M. Musci, M. Gerboles, E. Anklam, Contribution of dynamic headspace GC-MS analysis of aroma compounds to authenticity testing of honey, Food Chemistry, 72 (2001) 511-520.
13. A.C. Soria, I. Martinez-Castro, J. Sanz, Analysis of volatile composition of honey by solid phase microextraction and gas chromatography-mass spectrometry, Journal of Separation Science. 26 (2003) 793-801.
14. L.F. Cuevas-Glory, J.A. Pino, L.S. Santiago, E. Sauri-Duch, A review of volatile analytical methods for determining the botanical origin of honey, Food Chemistry. 103 (2007) 1032-1043.
15. E. Alissandrakis, P.A. Tarantalis, P.C. Harizanis, M. Polissiou, Aroma investigation of unifloral Greek Citrus honey using solid-phase microextraction coupled to gas chromatographic-mass spectrometric analysis, Food Chemistry, 100, 1 (2007) 396-404.
16. X. Yu, M. Zhao, F. Liu, S. Zeng, J. Hu. Identification of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one as a strong antioxidant in glucose–histidine Maillard reaction products, Food Research International, Volume 51, 1 (2013) 397–403.
17. M. Shimoda, Y. Wu, Y. Osajima, Aroma compounds from aqueous solution of haze (Rhus suceda´ nea) honey determined by absorptive column chromatography. Journal of Agricultural and Food Chemistry, 44 (1996) 3913–3918.
18. G. Bentivenga, M. D'Auria, P. Fedeli, G. Mauriello, R. Racioppi, SPME-GC-MS analysis of volatile organic compounds in honey from Basilicata. Evidence for the presence of pollutants from anthropogenic activities, International Journal of Food Science & Technology. 39 (2004) 1079-1086.
19. X. Yan, L. Yu-Hong, Comparison of 1.5% lidocaine and 0.5% ropivacaine epidural anesthesia combined with propofol general anesthesia guided by bispectral index.Journal of Zhejiang University. Science. B, 8, 6 (2007) 428-434.