GC-MS ANALYSIS OF TURKISH HONEYDEW HONEYS PRODUCTION OF *MARCHALINA HELLENICA* ON *PINUS BRUTIA*



ASLI ÖZKÖK[1], KADRİYE SORKUN[1], BEKİR SALİH[2]

aozkok@hacettepe.edu.tr kadriye@hacettepe.edu.tr bekir@hacettepe.edu.tr

[1]Hacettepe University Faculty of Sciences, Department of Biology, 06800, Beytepe-Ankara-Turkey

[2]Hacettepe University Faculty of Sciences, Department of Chemistry, 06800, Beytepe-Ankara-Turkey

Abstract

78 honey samples were collected from ten areas of Muğla city in western Turkey. These points were Merkez, Milas, Ortaca, Köyceğiz, Marmaris, Fethiye, Yatağan, Bodrum, Ula and Datça where honeydew beekeeping is practiced extensively, from September to November in the years of 2005 and 2006. All honey samples were examined under the light microscope. The Number of Honeydew Element (NHE) and the Number of Total Pollen (NTP) in these samples were also investigated. The quality of the samples was determined on the basis of the NHE/NTP ratio. Each honey sample having the NHE/NTP ratio higher than 4.5 was accepted as a high quality honeydew honey. Following microscopic studies, 50 honey samples were determined as honeydew honey samples and these samples were prepared for GC-MS analysis to screening the chemical composions.

As a result of organic chemical substances quantified by GC-MS of 50 honeydew honeys, it was found that the main organic compound groups were mainly aliphatic and aromatic aldehydes, aromatic alcohols, hydrocarbons with linear chains, acid esters with linear chains, aromatic hydrocarbons, flavanones, aromatic acids, aromatic acid esters, aromatic amines and other minor chemical substances.

INTRODUCTION

The origin of honeydew honey which includes pine honey as well is honeydew. Honeydew is the rectal secretion with high sugar content of insects feeding on phloem juice (Zander and Koch, 1994). Insects provide for their food needs from dense sugar solution in phloem juice and after sufficient intake excrete the rest. Microscopic fungus and algae emerge on sticky sugary excreta which may then pass to honey (D'Albore, 1997). Bees carry this dense sugary substance to their hives and transform it into honey. Honey produced by bees this way is called honeydew honey (Zander and Koch, 1994).

The origin of pine honey produced in Turkey *Pinus brutia* Ten. and it is produced by *Marchalina hellenica* (syn. *Monophlebus hellenicus*) which lives on it (Figure 1 and Figure 2). The habitat of this insect living on *Pinus brutia* Ten. consists only of Greece and Turkey (Santas, 1979). In Turkey *Marchalina hellenica* habitat covers Southern Marmara, Aegean and Western Mediterranean Regions (Gürkan, 1989).

Pine honey is produced in the world only in Turkey (90% of total amount) and in Greece (10% of total amount). In Turkey, a large part of honey produced by *Marchalina hellenica* from *Pinus brutia* and marketed commonly as "pine honey" comes from Muğla region (Şahin, 2000) (Figure 3-5).

Figure 1. *Pinus brutia* and *Marchalina hellenica* in Muğla

Figure 2. Mature *Marchalina hellenica* (Erlinghagen, 2001)

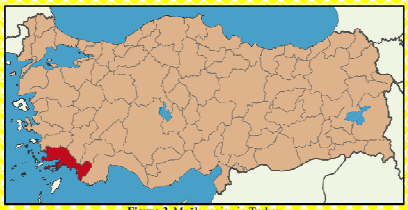

Figure 3. Muğla region in Turkey

Figure 4. Muğla-Fethiye Dead Sea region

Figure 5. Muğla-Marmaris region

Honeydew honey is generally characterized by honeydew elements (NHE) composed of microscopic micro algae, fungus seeds and spores. A honey with the ratio "number of honeydew elements"/"total number of pollens" is greater than 3 is considered as honeydew honey (Louveaux et al., 1978; Soria et al., 2004).

In this study NHE/NTP ratio higher than 4.5 was accepted as a high quality honeydew honey. Following microscopic studies, 50 honey samples were determined as honeydew honey samples (Figure 6) and these samples were prepared for GC-MS analysis to screening the chemical composions.

Figure 6. Microscopic appearance of one of the pine honey samples

MATERIAL 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 honeydew beekeeping is practiced extensively, from September to November in the years of between 2004 and 2006. In this study, suitable apiaries were chosen from villages (Figure 7-9). It was important that the villages are separated with enough distance and there are enough vegetation differences, which also represent Muğla statistically.

Figure 7. Muğla-Datça-Sındı village

Figure 8. Muğla-Milas-Pınar village

Figure 9. Muğla-Yatağan-Bencik village

Figure 10. Muğla-Fethiye-Ören village

Microscopic Analysis of Honey Samples

For microscopic analysis of the honey samples which were collected from 33 villages in the Muğla city, methods by Moar (1985) and Sorkun (2008) were followed. Honey preparations were examined with a Nikon Eclipse E400 microscope. As a result of microscopic analysis of the honey samples, 50 honey samples were selected because of the fact that their NHE to NTP ratios were bigger than 4,5. These honeydew honeys were found to be appropriate for GC-MS analysis study.

GC-MS Analysis of Honey Samples

Chemical analysis of honey samples have been made by GC-MS according to Barcarola et al, (1998); Radovic et al.,(2001); Soria et al.,(2003); Cuevas-Glory et al.,(2007) methods. According to this method 10 gr honey was weighed to a glass tube and 10 ml methanol was added. This mixture have been kept waiting until it was dry. After drying the mixture 10 ml ethanol was added and closed by lid. This mixture was waited 24 hours. After 24 hours this mixture was depleted another tube and waited ethanol dry. 0,5 ml ethanol was added and mixed with pipet. 2 µl mixture was injected to GC-MS. A GC 6890N from Hewlett-Packard (Palo Alto, CA, USA) coupled with mass detector (MS5973, Hewlett-Packard) was used for the analysis of honey samples. Experimental conditions of GC-MS system was as follows: DB 5MS column (30 mx 0.25mm 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 for 1 min at 50 °C and then increased to 150 °C with 10 °C/min heating ramp. After this period, temperature was kept at 150 °C for 2 min. Finally, temperature was increased to 280 with 20 °C/min. heating ramp and then kept at 280 °C for 30 min.

RESULTS

As a result of organic chemical substances quantified by GC-MS of 50 honeydew honeys, it was found that the main organic compound groups were mainly aliphatic and aromatic aldehydes, aromatic alcohols, hydrocarbons with linear chains, acid esters with linear chains, aromatic hydrocarbons, flavanones, aromatic acids, aromatic acid esters, and other minor chemical substances (Figure 11). Percent amounts of chemical substance has been shown in Table 1.

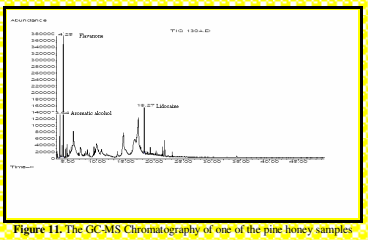

Figure 11. The GC-MS Chromatography of one of the pine honey samples

Table 1. GC-MS analysis results of pine honey samples

Sample no	Aromatic aldehydes	Aromatic Alcohols	Aldehydes	Flavanones	Hydrocarbons with linear chains	Aromatic hydrocarbons	Aromatic acid esters	Acid esters with linear chains	Aromatic Acids	Aromatic esters	Others	Lidocaine	2-Phenyl-2-(tipyl-acetoxy)propane
1	-	-	-	-	1.02	28.94	-	1.78	-	-	5.64	-	-
2	-	-	-	-	-	-	-	-	-	-	3.74	-	-
3	-	0.86	-	-	1.04	-	-	-	-	-	2.52	-	-
4	-	0.96	-	1.38	0.33	0.44	-	-	-	-	2.41	-	-
5	-	-	-	-	-	27.67	-	-	-	-	1.02	1.85	-
6	-	1.91	-	2.33	0.25	-	-	-	-	-	3.21	-	-
7	-	-	-	-	-	-	-	-	-	-	3.59	-	-
8	-	1.14	-	1.50	-	-	-	-	2.28	-	5.19	-	-
9	0.35	1.30	-	2.43	0.65	-	-	-	1.86	-	1.90	-	-
10	-	-	-	-	3.68	-	-	-	-	-	7.53	-	-
11	-	1.92	-	-	-	-	-	-	-	-	0.95	-	-
12	-	-	-	-	0.46	-	1.81	-	-	-	2.01	-	-
13	-	2.08	16.27	1.56	-	8.07	-	-	-	-	1.21	0.25	-
14	-	1.15	-	-	1.24	8.16	-	-	-	-	2.93	-	-
15	-	1.78	-	-	3.19	-	-	-	-	-	1.81	-	-
16	0.47	0.94	7.51	-	0.36	-	-	0.33	0.69	-	3.86	-	-
17	-	1.06	-	1.59	1.03	-	-	-	0.98	-	2.74	-	-
18	0.81	0.90	-	-	1.67	1.03	-	0.62	0.65	-	2.76	-	-
19	0.56	1.57	-	-	1.26	-	-	0.98	1.31	-	4.88	-	-
20	-	1.28	-	1.74	0.53	-	-	-	-	-	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	-	-	-	-	-	-	-	-	0.80	-	-
24	-	1.48	-	2.38	2.33	-	-	-	-	-	2.86	-	-
25	-	-	-	-	-	0.75	-	-	-	-	0.98	-	-
26	-	1.08	-	1.64	-	-	-	-	-	-	2.52	-	-
27	-	1.33	-	2.77	0.38	4.86	-	0.44	1.47	-	2.50	-	-
28	-	1.40	-	2.47	1.07	-	-	-	-	-	8.86	-	-
29	0.51	1.78	5.80	2.01	-	-	-	-	0.63	-	2.91	-	-
30	-	1.38	-	2.36	2.74	-	-	-	-	-	3.36	-	-
31	-	0.93	-	-	3.30	-	-	-	-	1.24	2.82	-	-
32	-	-	-	-	0.49	-	-	-	-	-	3.68	-	-
33	0.49	1.10	-	2.14	-	-	-	-	0.64	-	2.22	-	-
34	-	1.20	-	2.01	-	-	-	-	-	0.50	3.10	-	-
35	-	1.29	13.83	3.31	1.43	-	-	0.36	-	-	1.55	-	-
36	-	1.57	-	-	1.64	-	-	-	-	-	2.04	-	-
37	0.73	1.80	-	-	0.44	-	-	0.12	0.76	-	2.63	-	-
38	-	1.49	-	-	1.44	3.19	-	-	-	-	2.35	-	-
39	-	1.05	-	2.07	2.10	-	-	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	-	-	1.67	13.53	-	-	-	-	1.78	-	-
43	-	1.35	-	2.37	1.47	-	-	-	1.12	-	4.48	-	-
44	-	-	-	-	-	9.75	-	-	-	3.46	3.35	-	-
45	-	1.36	-	2.82	-	-	-	-	-	-	2.34	-	-
46	-	1.64	-	2.57	1.19	-	-	-	-	-	4.67	-	-
47	-	1.35	-	2.26	-	-	-	0.52	-	-	3.46	-	-
48	-	1.52	-	2.54	-	-	-	1.13	-	-	3.13	-	-
49	-	1.17	-	-	2.87	-	-	1.01	-	-	2.34	-	-
50	1.33	0.57	-	1.98	-	-	-	-	5.92	-	2.09	-	-

DISCUSSION

At this study generally aromatic aldehydes and aromatic alcohols have been identified at all honey samples. Radovic et al. (2001) also determined aromatic aldehydes and aromatic alcohols at honey samples. Alissandrakis et al. (2007) also found aromatic alcohols at honey samples.

Hydrocarbons with linear chains were found at 1,3,4,6,9,10,12,14,15,16,17,18,19,20,21,22,24,27,28,30,31,32,35,36,37,38,39,40,41,42,43,46 and 49 honey samples. Bentivenga et al. (2004) also determined hydrocarbons with linear chains at honey and beeswax.

Aromatic hydrocarbons shows environmental pollution. These kind of components especially indicate environmental pollution at water. (Bentivenga et al., 2004). It has been determined aromatic hydrocarbons at 1,4,5,13,14,18,25,27,38,40,42,44 honey samples.

Antioxidant activity is the ability and potential of honey in reducing oxidative reactions within the food systems and human health. Notably, these oxidative reactions can cause deleterious reactions in food products (e.g. lipid oxidation in meat, and enzymic browning in fruits and vegetables) and adverse health effects, such as chronic diseases and cancers (Gheldof and Engeseth, 2002). The antioxidants that naturally occur in honey contribute to antioxidant capacity (Nicholls and Miraglio, 2003). It has been found flavanones at honey sample numbers 4,6,8,9,13,17,20,21,22,24,26,27,28,29,30,33,34,35,39,43,45,46,47,48,50. Sample 35 has the highest flavanone amount with %3,31. Shimoda et al. (1996), also determined flavanones at honey samples.

Lidocaine, which is used local anesthetic component, was found at all honey samples. Yan and Yu-Hong, (2007) also was determined this component at honey samples.

This study showed that the 50 samples of Muğla Turkish Pine Honey contained a lot of different components. Some of them good quality but some of them bad. So it is need to make different experiments and studies.

ACKNOWLEDGEMENTS

We are indebted to the TUBITAK for financial support, and also to MUĞLA ARI YETİŞTİRİCİLERİ BİRLİĞİ and local people who helped us during our fieldwork.

REFERENCES

Alissandrakis, E., Tarantilis, P. A., Harizanis, P. C. Polissiou, M., 2007. Aroma investigation of unifloral Greek citrus honey using solid-phase microextraction coupled to gas chromatography–mass spectrometric analysis. Food Chemistry, 100 (1), 396-404.

Barcarola, R., Centeleghe, M., Zanella, P. and Cosi, L. S., 1998. 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, 11-21 p.

Bentivenga, G., D'Auria, M., Fedeli, P., Mauriello, G., Racioppi, R., 2004. 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 (10), pp. 1079-1086(8).

Cuevas-Glory, L.F., Pino, J. A., Santiago, L. S. and Sauri-Duch, E., 2007. A review of volatile analytical methods for determining the botanical origin of honey. Food Chemistry, 103: 1032-1043.

D'Albore, G. R., 1997. Textbook of Melissopalynology. Aprilcodila Publishing House, Bucharest, 308 p.

Erlinghagen, F., 2001. Portrait of an insect: Marchalina hellenica Genn.(Sternorrhyncha: Coccoidea: Margarodidae), important producer of honeydew in Greece. Apiacta, 36: 131-137.

Gheldof H., Engeseth N. J. (2002). Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. Journal of Agricultural and Food Chemistry, 50 (10), 3050-3055.

Gürkan, B., 1989. Çam pamuklu koşnili *Marchalina hellenica* (Genadius) 'nin Biyo-Ekolojisi ve Popülasyon Dinamiği. Doktora Tezi, Ankara.

Louveaux, J., Maurizio, A., Vorwohl, G., 1978. International Commission for Bee Botany of IUBS. Methods of Melissopalynology. Bee World, 59, 139-157.

Moar, N. T., 1985. Pollen analysis of New Zealand honey. New Zealand Journal of Agricultural Research, Vol. 28, 38-70.

Nicholls, J., Miraglio A. M. (2003). Honey and healthy diets. Cereal Foods World, vol. 48, no. 5, pp. 116-119.

Radovic, B. S., Careri, M., Mangia, A., Musci, M., Gerboles, M. and Anklam, E., 2001. Contribution of dynamic headspace GC-MS analysis of aroma compounds to authenticity testing of honey. Food Chemistry, 72, pp. 511-520.

Santas, L. A., 1979. *Marchalina hellenica*: An Important Insect for Apiculture of Greece. The XXVIIth International Congress of Apiculture of Apimondia, Athens, 419-422.

Shimoda, M., Wu, Y., Osajima, Y. 1996. Aroma compounds from aqueous solution of haze (Rhus succedanea) honey determined by headspace column chromatography. Journal of Agricultural and Food Chemistry, 44 (3), 3933-3938.

Soria, A. C., Gonzalez, M., Lorenzo, C., Martinez-Castro, I., Sanz, J., 2004. Characterization of artisanal honeys from Madrid (Central Spain) on the basis of their melissopalynological, physicochemical and volatile composition data. Food Chemistry, 85, 121-130.

Soria A. C., Martinez-Castro, I. and Sanz, J., 2003. Analysis of volatile composition of honey by solid phase microextraction and gas chromatography–mass spectrometry. Journal of Separation Science 26, 793-801.

Sorkun, K., 2008. Türkiye'nin Nektar-Bal-Arı Bitkileri ve Ballar, Palme Yayıncılık, 343s.

Şahin, A., 2000. Marmaris-Muğla Yöresinde Üretilen Çam (Balkanası Mikrolojisi, Analiz ve Organoleptik Özelliklerinin Saptanması Yüksek Lisans Tezi, Adana.

Yan, Y., Yu-Hong, L., 2007. Comparison of 15% lidocaine and 0.5% ropivacaine epidural anesthesia combined with propofol general anesthesia guided by bispectral index. *Journal of Zhejiang University, Science. B*, 8 (6), p.428-434. Jun.

Zander, E., Koch, A., 1994. Der Honig. Ehgen Ulmer Verlag, Stuttgart, 201 ff.