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ORIGINAL PAPER

Characterization of volatile compounds of Turkish pine honeys from different regions and classification with chemometric studies

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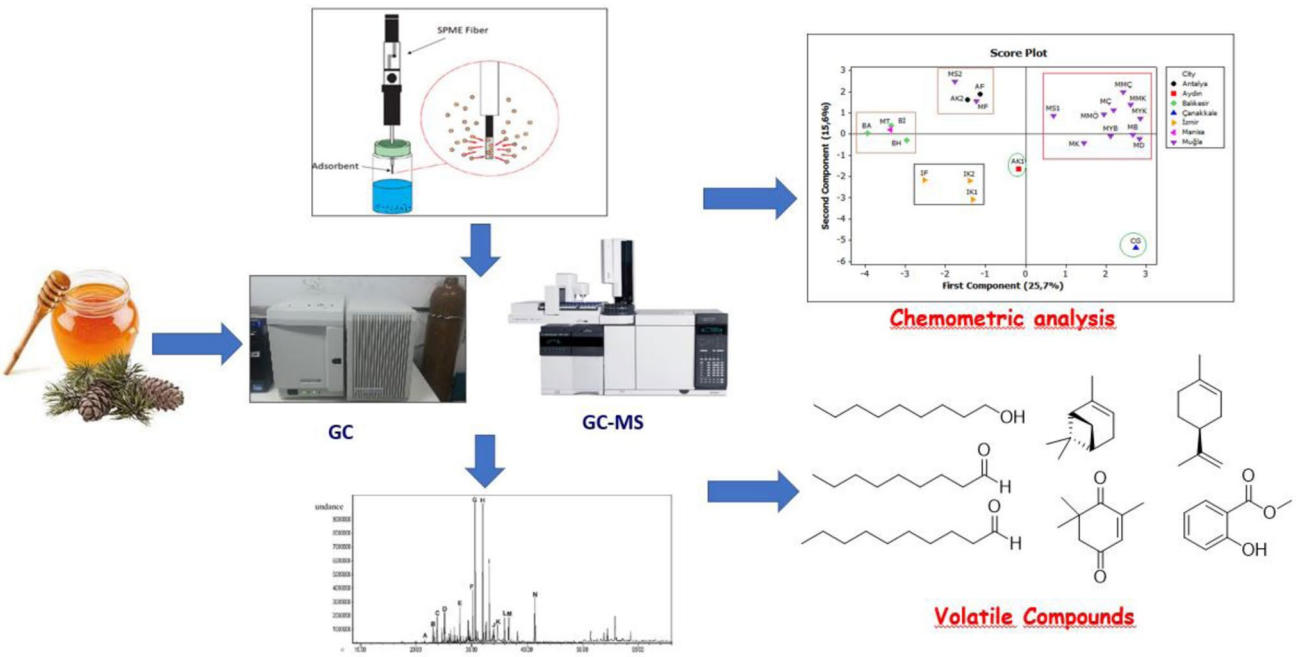
Abstract

Pine honey is highly appreciated by consumers because of its non-crystallization, appearance, color, taste, and unique aroma. The objective of this study was to investigate the volatile compounds of pine honeys from 23 stations from 7 regions in Turkey's Aegean and Mediterranean regions. Solid-phase microextraction, followed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) systems were used to determine volatile compounds qualitatively and quantitatively. A total of 32 volatile compounds were identified and octanol, nonanol, 4,4,7-α-trimethyl 2,4,5,6,7-α-hexahydro-1-benzofuran-2-yl-methanol, benzaldehyde, octanal, phenylacetaldehyde, nonanal, decanal, 2-nonanone, 4-oxoisophorone, methyl salicylate, α-pinene, β-pinene, limonene, *cis*-linalool oxide, borneol, 1,8-cineole, and β-damascenone were found to be common volatile compounds. The classification based on the geographical origin of pine honey samples was determined using principal component analyses (PCA) and hierarchical clustering analyses (HCA). These results revealed that nonanal, nonanol, octanol, decanal, phenylacetaldehyde, benzaldehyde, octanal, α-pinene, 4-oxoisophorone, methyl salicylate, isopropyl myristate, limonene, and β-damascenone could be used as marker compounds in Turkish pine honeys.

Graphic abstract



Keywords Pine honey · Volatile compounds · SPME · GC–MS · Geographic origin · Chemometrics

Extended author information available on the last page of the article

Introduction

Honey is a natural food that is collected by honey bees (*Apis mellifera*), is made up of the nectar secreted from the flowers or other living parts of the plants and the substances secreted by some insects living on the plant [1, 2]. Honey has a high nutritional value and, unlike many foods, does not spoil in a short time, it can be stored for a long time without the need for cold storage [3, 4].

While soft scaly insects such as aphids, whiteflies, *Marchalina hellenica*, *Lachnus iliciphilus* and *Thelaxes dryophila* feed by sucking the sap of some plants, the feces they leave to the environment are an important source of nectar for the honey bee. *Marchalina hellenica* (syn. *Monophlebus hellenicus*) (Coccoidea: Homoptera) mostly lives as a parasite in some pine tree species such as *Pinus brutia* and *P. pinea* in the Aegean and Mediterranean regions [5, 6]. Pine honey is a type of honey that is obtained upon the collections of honeydew which contains high sugar content secreted by the insect *Marchalina hellenica* feeding on the sap of pine trees pertaining to the species *P. brutia* and *P. halepensis* by honey bees [7]. Pine honey is distinguished from other honeys with its unique smell, chemistry, and taste properties. It is preferred by consumers because of its thick consistency, high mineral content, low sweetness rate, and less tendency to freeze and crystallize [8].

The discrimination between blossom and honeydew honeys has been an interesting research area among scientists for years. Several researches have been carried on the physicochemical characteristics and melyssopalynological analysis of honeydew and blossom honey, as well as their distinction [9–12]. Although the microscopic analysis of pine honey is similar to the pollen analysis performed in blossom honey, the method is different and the density of fungal spores, hyphae and algae is determined in addition to the pollen found in honey. Ratio between honeydew elements (fungal spores, hyphae, algae and wax elements of honeydew-producing insects) and pollen grains (HDE/P) as an indicator of the difference between blossom and honeydew honeys. Honeydew honey is described as honey with HDE/P ratio greater than three according to melyssopalynological analysis [7, 12, 13].

Turkey located second after China in the world honey production [14], has 90% of world production of pine honey [15, 16]. Pine honey is produced on average 30,000 tonnes each year in Turkey and a very large portion of this amount is exported to European countries, especially Germany [7, 16, 17]. Turkey and Greece are two well-known countries for the production of pine honey due to favorable climatic conditions and relative humidity [7]. Around the Muğla region is the major pine honey production area compared with other production areas in Turkey. Approximately 10,000 beekeepers and forty thousand tons of pine honey are produced annually in these regions. Pine honey is produced in 2 or 3 different periods between September and December, depending on the climate. Since pine honey does not crystallize easily, it is not subjected to any heating process and always preserves the quality of raw honey. Pine honey, one of the dark-colored honeys, has high phenolic content and high antioxidant, anti-inflammatory, and antimicrobial properties [18–20].

In honey, volatile compounds synthesized by various biosynthetic routes are extracted using a wide variety of methods and divided into chemical subcategories, such as aldehydes, ketones, acids, alcohols, hydrocarbons, norisoprenoids, terpenes, benzene compounds, furan and pyran derivatives. These volatile compounds represent a honey fingerprint, providing valuable information about the botanical and geographical origin of honey [21]. The identification of volatile compounds in honey helps to standardize the quality of honey, to prevent fraudulent mislabelling of poor-quality products and also to support the authenticity of the product. In addition, it has been reported that some volatile compounds contained in honey contribute to the general biomedical properties, such as antioxidant, anti-inflammatory, antimicrobial and immune-modulatory effects of honey [22]. There are some studies related to aroma compounds of different type of honeys but studies on pine honeys very limited. Ozcan-Sinir et al. [23] investigated volatile compounds of some multifloral and monofloral honeys including pine honeys using selected-ion flow-tube mass spectrometry technique. In another study, the aroma compounds of pine honeys from two locations were determined by the solvent-assisted flavour evaporation method [24]. Silici [25] characterized volatile compounds of pine honeys with SPME–GC/MS method and mentioned that nonanal, benzene, 4-hexen-3-ol, alpha-pinene, and 2-heptanone were recognized to be markers of the pine honey. In addition, Bayraktar and Onoğur [26] reported volatile compounds of pine honeys from three collection area and found that nonanal, nonanol, decanal, octanal, 16-oxosalutaridine, dodecanal, nonadecane, and pentadecane were common compounds in pine honeys. Tananaki et al. [6] published a comparative analysis of volatile and semi-volatile compounds from Turkish and Greek Pine honeys with purge and trap GC–MS systems. Karabagias et al. [18] characterized and classified Greek pine honeys according to geographical origin using volatile compounds and physicochemical properties. Thirty-nine pine honey samples were used from four different regions in Greece. Nine selected volatile compounds and eleven physicochemical parameters were used for the classification of pine honey samples. In a different study, the volatile fraction of 34 commercial thyme honeys from Morocco, Egypt, Spain, and Greece were investigated according to geographic

origin using key volatile compounds along with chemometrics [27]. Classification and discrimination between the different types of honeys (chestnut, cotton, citrus, fir, heather, pine, and thyme) from Greece were investigated by Aliferis et al. [28].

There are few studies on aroma components of the pine honey produced in Turkey, and they are made out of samples taken at random from several localities. In addition, there is no research on the volatile components of the pine honey produced in regions where dense pine honey in Turkey. In the present research, volatile compounds of pine honeys from 23 stations from 7 regions in Turkey's Aegean and Mediterranean regions are produced periodically for 2 years under the control of technical staff in the Ministry of Agriculture and Forestry, and the Turkish Association of Beekeepers were investigated. In addition, the volatile marker compounds of Turkish pine honey with chemometric studies were also determined in this study.

Materials and methods

Honey material

A total of 23 pine honeys produced at different locations in Turkey were provided by the Turkish Association of Beekeepers (TAB). Samples were obtained during the 2016 and 2017 harvests. Geographical location of pine honey samples is given in Fig. 1. Codes and locations of pine honeys are presented in Table 1. The melyssopalynological analysis was

Table 1 Sample code and production locations of the studied pine honeys

No	Sample code	Location
1	CG	Çanakkale-Gelibolu
2	Bİ	Balıkesir-İvrindi
3	BA	Balıkesir-Altınoluk
4	BH	Balıkesir-Havran
5	MT	Manisa-Turgutlu
6	IK1	İzmir-Kemalpaşa
7	IF	İzmir-Foça
8	IK2	İzmir-Karşıyaka
9	AK1	Aydın-Kuşadası
10	MB	Muğla-Bodrum
11	MMO	Muğla-Milas-Ören
12	MMC	Muğla-Milas-Çukurköy
13	MMK	Muğla-Milas-Kayaderesi
14	MYK	Muğla-Yatağan-Kozağaç
15	MYB	Muğla-Yatağan-Bencik
16	MK	Muğla-Menteşe-Kıran
17	MS1	Muğla-Menteşe-Sarnıç
18	MC	Muğla-Marmaris-Çetibeli
19	MD	Muğla-Datça
20	MF	Muğla-Fethiye
21	MS2	Muğla-Seydikemer
22	AK2	Antalya-Kaş
23	AF	Antalya-Finike

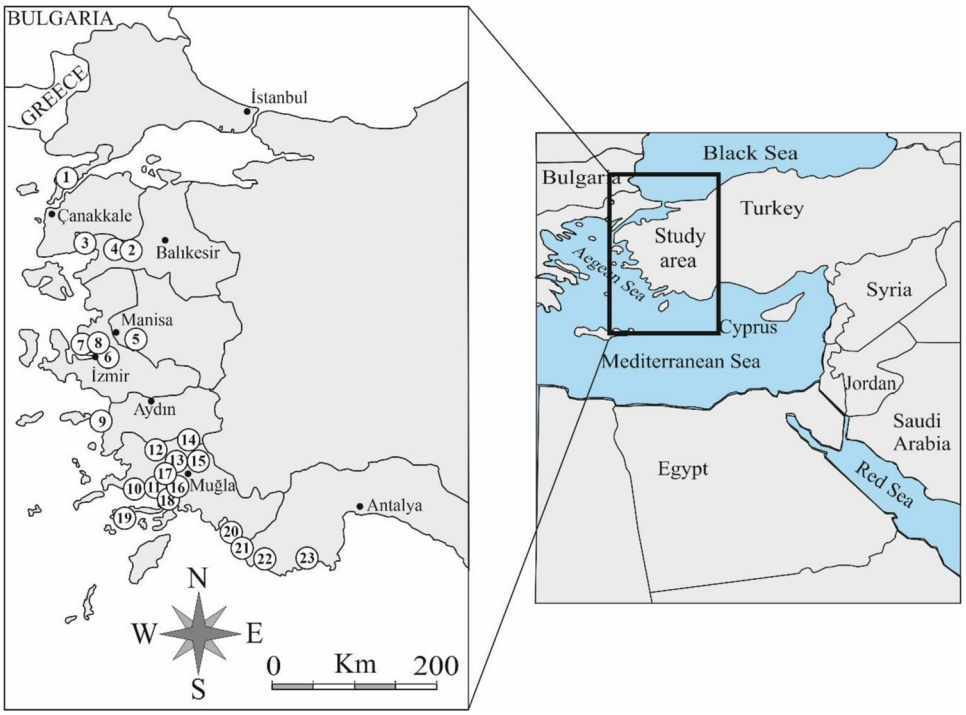


Fig. 1 Geographical location of pine honey samples

used to verify that the honey samples were as honeydew honey by Muğla University-Food Analysis Application and Research Center. The honeydew element (HDE) index was calculated to confirm the honeydew origin of honeys. The quantity of HDE (fungal and algal elements) divided by the number of pollen grains (HDE/P) is the ratio [7, 12, 13]. According to the results of melyssopalynological analysis, honey samples with honeydew elements and a number of pollen grains ratio HDE/P > 3 were used in the study.

Extraction of the volatile compounds

Volatile compounds of pine honey samples were investigated by solid-phase microextraction (SPME), followed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) systems. Before analysis, the fiber was preconditioned and thermally cleaned in the injection port of a gas chromatograph according to the instructions provided by the supplier. Analyses were carried out by weighing 5 g of honey sample in 20 mL vials with adding 5 mL of a 20% sodium chloride solution. Samples were maintained and magnetically stirred for 30 min at 50 °C to allow equilibration. Thus, the fiber was introduced into the vial and exposed to the headspace under the sample for 30 min. When the extraction step was completed, the SPME fiber was removed from the vial and inserted into the injection port of the GC–MS for thermal desorption of the volatile compounds [29].

GC–FID and GC–MS analyses

For GC–FID analyses, flame ionization detector (FID) and Rxi-5Sil MS (Restek) fused silica non-polar capillary column (30 m × 0.25 I.D., film thickness 0.25 µm) were used. The detector and injector temperatures were adjusted to 270 and 250 °C, respectively. Helium was used as carrier gas with a 1.4 mL/min flow rate. The initial oven temperature was held at 60 °C for 5 min, then increased up to 280 °C with 4 °C/min increments and kept at this temperature for 5 min. The Class GC10 GC computer program determined the percentage composition of the volatile compounds.

GC–MS analyses were performed using an ion trap MS spectrometer (Varian) and an Rxi-5Sil MS (Restek) fused silica non-polar capillary column (30 m × 0.25 mm I.D., film thickness 0.25 µm). Samples were injected in splitless mode and carrier gas was Helium with a 1.4 mL/min flow rate. The injector port and MS transfer line temperatures were 220 and 290 °C, respectively. The ion source temperature was at 200 °C. EI–MS measurements were taken at 70 eV ionization energy. Mass range was from *m/z* 28 to 650 amu. Scan time was 0.5 s with 0.1 s interscan delays. The oven temperature was maintained at 60 °C for 5 min then increased up to 280 °C with 4 °C/min increments and kept at this temperature for 5 min. Identification of volatile compounds was based on GC retention indices and computer matching with the Wiley, ADAMS, and NIST 08 MS databases, as well as by comparison of the fragmentation patterns of the mass spectra reported in the literature and whenever possible, by co-injection with standard compounds [30].

Statistical analysis

All statistical calculations for chemometric studies of the volatile compounds of the pine honey samples by principal component analysis (PCA) and hierarchical cluster analysis (HCA) were carried out by MINITAB 16.0 software. Cluster analysis was employed to get hierarchical relations using the Ward Linkage method and Euclidean distance.

Results and discussion

Volatile compounds

Volatile compounds of 23 pine honey samples produced at different locations in Turkey were analyzed using SPME–GC–MS. The results of volatile compounds of pine honey samples are given in Table 2 as a percentage (%). The 32 compounds including alcohols, aldehydes, ketones, esters, acids, and terpenes were detected. These compounds have different percentages based on the geographical origin of the honey. The decreasing order of volatile compounds classes according to the total volatile compounds for 7 different regions was: aldehydes > terpenes > alcohols > ketones > esters for the Çanakkale pine honey sample; aldehydes > alcohols > terpenes > ketones > esters for the Balikesir and Manisa pine honey samples; alcohols > terpenes > aldehydes > ketones > esters > acids for the İzmir pine honey samples; aldehydes > alcohols > terpenes > esters > ketones > acids for the Aydın pine honey sample; aldehydes > alcohols > terpenes > esters > ketones > acids for the Antalya pine honey sample. Respective order of Muğla pine honey samples was: aldehydes > alcohols > terpenes > esters > ketones for the MB, MMO, MMC, MMK, MYK, and MYB regions; aldehydes > terpenes > alcohols > ketones > esters for the MK region; aldehydes > alcohols > terpenes > ketones > esters for the MS1 region; aldehydes > alcohols > terpenes > esters > ketones > acids for the MC, MF and MS2 regions; aldehydes > terpenes > alcohols > ketones > esters > acids for the MD region.

Alcohols were one of the most important volatile compounds responsible for the characteristic fragrance of pine honey [24]. Three alcohol compounds such as octanol, nonanol and 4,4,7-α-trimethyl 2,4,5,6,7-α-hexahydro-1-benzofuran-2-yl-methanol were detected in this study.

Table 2 Volatile compounds of pine honeys (%)

Compounds	RI[a]	LRI[b]	ID[c]	CG	BI	BA	BH	MT	IK1	IF	IK2	AK1	MB	MMO	MMC
Alcohols															
Octanol	1076	1063	Co-GC, LRI, MS	3.94	1.47	1.05	1.62	1.39	0.38	0.28	0.33	0.18	2.21	2.40	1.96
Nonanol	1168	1165	Co-GC, LRI, MS	8.45	23.43	22.84	15.70	26.71	14.42	14.54	7.48	19.47	13.98	20.27	19.52
4,4,7-*α*-Trimethyl 2,4,5,6,7-*α*-hexahydro-1-benzofuran-2-yl-methanol	1280	1275	LRI, MS	0.73	0.25	0.58	0.92	0.28	1.54	1.21	1.38	2.33	3.71	2.24	1.09
Total				13.12	25.15	24.47	18.24	28.38	16.34	16.03	9.19	21.98	19.90	24.91	22.57
Aldehydes															
Benzaldehyde	961	952	Co-GC, LRI, MS	5.01	0.38	0.85	0.95	0.28	0.65	0.24	0.35	1.55	0.48	0.49	0.75
Octanal	980	988	Co-GC, LRI, MS	2.98	0.39	0.68	0.90	0.78	3.01	3.07	3.66	2.25	2.80	2.05	4.44
[*E*]-2-octenal	1061	1059	LRI, MS	–	3.45	–	–	0.15	–	–	–	–	1.65	–	1.20
Phenyl acetaldehyde	1067	1064	Co-GC, LRI, MS	3.52	0.24	0.95	1.05	0.19	0.23	0.24	0.48	1.55	1.45	0.97	1.19
Nonanal	1082	1088	Co-GC, LRI, MS	21.90	44.77	46.92	52.39	47.30	47.22	52.57	61.17	45.61	44.55	36.03	36.65
Lilac aldehyde A	1148	1145	LRI, MS	–	–	–	–	–	–	–	–	3.87	–	–	–
Lilac aldehyde B	1155	1151	LRI, MS	–	–	–	–	–	1.31	1.14	1.21	2.97	–	–	–
Safranal	1188	1196	LRI, MS	0.33	4.13	1.58	–	0.15	0.24	0.29	0.33	–	1.47	3.51	3.04
Decanal	1205	1201	LRI, MS	9.07	7.94	7.36	6.20	5.03	14.67	15.08	10.34	6.46	5.62	6.07	6.15
Total				42.81	61.30	58.34	61.49	53.88	67.33	72.63	77.54	64.26	58.02	49.07	53.42
Ketones															
2-Nonanone	1079	1087	Co-GC, LRI, MS	0.38	0.59	0.68	1.05	0.18	0.24	0.26	0.33	0.15	0.78	1.21	0.91
α-Isophorone	1121	1118	LRI, MS	0.15	0.39	2.06	1.15	0.91	0.28	0.10	0.15	–	–	–	–
4-Oxoisophorone	1140	1136	LRI, MS	4.81	1.27	1.72	1.92	6.17	2.74	1.99	2.62	0.25	1.68	4.13	3.38
Total				5.34	2.25	4.46	4.12	7.26	3.26	2.35	3.10	0.40	2.46	5.34	4.29
Esters															
Methyl salicylate	1177	1190	LRI, MS	2.53	0.69	0.78	0.97	0.27	1.15	0.47	1.09	2.95	4.26	3.81	3.01
Isopropyl myristate	1788	1802	LRI, MS	–	–	–	–	–	–	–	0.64	0.36	1.42	1.63	6.78
Total				2.53	0.69	0.78	0.97	0.27	1.15	0.47	1.73	3.31	5.68	5.44	9.79
Acids															
Nonanoic acid	1277	1267	Co-GC, LRI, MS	–	–	–	–	–	0.10	0.14	0.33	0.11	–	–	–
Total				–	–	–	–	–	0.10	0.14	0.33	0.11	–	–	–
Terpenes															
α-Pinene	940	932	Co-GC, LRI, MS	10.45	0.40	0.95	1.92	0.45	0.62	0.44	0.48	2.05	2.32	1.10	1.34
β-Pinene	975	974	Co-GC, LRI, MS	0.57	0.18	0.59	1.05	0.15	0.50	0.26	0.33	0.39	0.59	0.68	0.77
cis-*β*-Ocimene	1037	1032	LRI, MS	–	–	–	–	–	–	–	–	–	–	–	–
p-Cymene	1048	1044	Co-GC, LRI, MS	1.09	0.18	0.48	1.05	0.15	0.24	0.23	0.33	–	–	0.43	0.43
Limonene	1052	1050	Co-GC, LRI, MS	9.16	0.15	0.58	0.75	0.28	0.26	0.31	0.32	0.26	0.35	0.93	0.42
1,8-Cineole	1057	1056	Co-GC, LRI, MS	2.57	0.08	–	–	0.88	2.60	2.23	2.97	2.35	3.71	3.26	1.71
cis-Linalool oxide	1072	1067	Co-GC, LRI, MS	3.03	0.24	0.98	0.92	0.18	0.51	0.16	0.15	0.67	0.92	0.93	0.85

Table 2 (continued)

Compounds	RI[a]	LRI[b]	ID[c]	CG	BI	BA	BH	MT	IK1	IF	IK2	AK1	MB	MMO	MMC
Borneol	1161	1165	Co-GC, LRI, MS	4.39	5.21	2.38	3.18	4.22	2.71	1.81	2.32	2.04	3.78	4.15	2.73
Myrtenol	1185	1194	Co-GC, LRI, MS	2.53	0.21	0.51	1.05	0.45	1.76	0.28	0.15	0.10	0.48	0.61	–
Eucarvone	1243	1236	Co-GC, LRI, MS	–	–	–	–	–	0.98	0.26	0.15	1.05	0.48	1.03	0.48
Bornyl acetate	1283	1284	Co-GC, LRI, MS	–	–	–	–	–	–	–	–	–	0.63	–	–
Thymol	1292	1289	Co-GC, LRI, MS	0.21	0.65	2.02	0.45	–	–	–	–	0.95	–	–	–
β-Damascenone	1370	1361	LRI, MS	2.20	3.31	3.46	4.81	3.45	1.64	2.40	0.91	0.08	0.05	1.64	0.42
trans-Geranyl acetone	1433	1453	LRI, MS	–	–	–	–	–	–	–	–	–	0.63	0.48	0.78
Total				36.20	10.61	11.95	15.18	10.21	11.82	8.38	8.11	9.94	13.94	15.24	9.93

Compounds	RI[a]	LRI[b]	ID[c]	MMK	MYK	MYB	MK	MS1	MC	MD	MF	MS2	AK2	AF
Alcohols														
Octanol	1076	1063	Co-GC, LRI, MS	2.41	2.37	1.88	1.51	1.66	1.88	3.13	0.77	0.46	0.91	1.02
Nonanol	1168	1165	C Co-GC, LRI, MS	20.58	23.11	15.48	12.35	20.11	26.35	11.56	19.47	28.78	21.44	28.01
4,4,7-*α*-Timethyl 2,4,5,6,7-*α*-hexahydro-1-benzofuran-2-yl-methanol	1280	1275	LRI, MS	1.30	1.92	2.43	0.65	0.98	1.11	1.96	0.43	0.42	0.85	0.87
Total				24.29	27.40	19.79	14.51	22.75	29.34	16.65	20.67	29.66	23.20	29.90
Aldehydes														
Benzaldehyde	961	952	Co-GC, LRI, MS	0.52	0.60	2.51	1.03	0.99	1.30	1.03	0.62	0.41	1.73	1.09
Octanal	980	988	Co-GC, LRI, MS	2.78	3.57	3.61	3.82	1.76	2.54	3.61	0.54	0.46	0.88	0.94
[*E*]-2-octenal	1061	1059	LRI, MS	0.51	–	–	–	–	–	–	–	–	–	–
Phenyl acetaldehyde	1067	1064	Co-GC, LRI, MS	1.28	1.35	0.60	0.63	0.42	0.73	1.01	0.58	0.43	0.89	1.14
Nonanal	1082	1088	Co-GC, LRI, MS	37.10	28.51	41.55	48.12	38.09	28.00	30.45	31.44	42.21	48.57	47.15
Lilac aldehyde B	1148	1145	LRI, MS	–	–	–	–	–	–	–	–	–	–	–
Lilac aldehyde A	1155	1151	LRI, MS	–	–	–	–	–	–	–	–	–	–	–
Safranal	1188	1196	LRI, MS	0.31	3.43	0.93	1.39	4.56	3.19	11.02	21.05	5.25	0.88	1.10
Decanal	1205	1201	LRI, MS	3.62	6.89	5.40	5.18	4.32	6.19	6.24	5.98	4.83	4.24	3.81
Total				46.12	44.35	54.60	60.17	50.14	41.95	53.36	60.21	53.59	57.19	55.23
Ketones			Co-GC, LRI, MS											
2-Nonanone	1079	1087	LRI, MS	0.85	1.36	1.24	1.13	1.07	0.95	2.00	0.84	0.91	0.82	1.04
α-Isophorone	1121	1118	LRI, MS	–	–	–	–	–	–	–	–	–	–	–
4-Oxoisophorone	1140	1136		3.28	3.69	3.29	3.58	4.31	2.46	2.21	2.31	1.68	1.88	1.94
Total				4.13	5.05	4.53	4.71	5.38	3.41	4.21	3.15	2.59	2.70	2.98
Esters			LRI, MS											
Methyl salicylate	1177	1190	LRI, MS	3.45	4.82	4.64	2.58	2.84	5.10	2.86	2.98	3.38	1.88	2.14
Isopropyl myristate	1788	1802		2.23	1.66	1.15	0.95	0.79	1.17	0.85	0.97	1.51	0.91	1.14
Total				5.68	6.48	5.79	3.53	3.63	6.27	3.71	3.95	4.89	2.79	3.28

Table 2 (continued)

Compounds	RI[a]	LRI[b]	ID[c]	MMK	MYK	MYB	MK	MS1	MC	MD	MF	MS2	AK2	AF
Acids														
Nonanoic acid	1277	1267	Co-GC, LRI, MS	–	–	–	–	–	1.22	0.31	0.39	0.22	0.15	0.17
Total				–	–	–	–	–	1.22	0.31	0.39	0.22	0.15	0.17
Terpenes														
α-Pinene	940	932	Co-GC, LRI, MS	9.46	3.61	2.15	3.59	3.16	3.72	4.59	1.74	1.46	1.85	1.14
β-Pinene	975	974	Co-GC, LRI, MS	0.57	0.18	0.79	0.65	0.45	0.93	1.29	0.65	0.38	1.95	1.15
cis-*β*-Ocimene	1037	1032	LRI, MS	–	–	–	–	0.48	0.15	1.93	0.15	0.17	0.10	0.10
p-Cymene	1048	1044	Co-GC, LRI, MS	0.30	0.22	0.91	0.12	0.46	0.91	0.50	0.56	0.30	0.88	1.04
Limonene	1052	1050	Co-GC, LRI, MS	0.31	0.70	3.65	2.85	0.48	1.42	2.16	0.56	0.29	0.85	0.95
1,8-Cineole	1057	1056	Co-GC, LRI, MS	1.98	3.24	2.38	4.26	2.59	4.19	2.67	0.95	0.72	0.45	1.05
cis-Linalool oxide	1072	1067	Co-GC, LRI, MS	1.54	2.23	0.86	0.65	0.58	0.27	0.15	0.54	0.69	0.87	0.46
Borneol	1161	1165	Co-GC, LRI, MS	2.90	4.47	3.37	3.17	4.76	4.34	3.28	0.95	0.87	2.01	1.14
Myrtenol	1185	1194	Co-GC, LRI, MS	–	0.16	0.45	0.60	0.21	0.10	1.73	0.18	0.14	0.10	0.12
Eucarvone	1243	1236	Co-GC, LRI, MS	0.31	0.50	0.18	0.12	0.58	0.47	0.79	0.95	0.43	0.88	–
Bornyl acetate	1283	1284	Co-GC, LRI, MS	1.13	–	–	–	–	–	–	–	–	–	–
Thymol	1292	1289	Co-GC, LRI, MS	–	–	–	–	1.60	–	–	–	–	–	–
β-Damascenone	1370	1361	LRI, MS	0.31	0.95	0.29	0.64	2.46	0.96	2.43	4.23	3.42	3.93	1.15
trans-Geranyl acetone	1433	1453	LRI, MS	0.97	0.46	0.26	0.43	0.29	0.35	0.24	0.17	0.18	0.10	0.14
Total				19.78	16.72	15.29	17.08	18.10	17.81	21.76	11.63	9.05	13.97	8.44

Percentage concentration

[a]Kovats index on Rxi-5Sil MS fused silica column

[b]LRI: Linear retention index taken from Adams (2007)

[c]Identification: Co-I: co-injection: based on comparison with standard compounds; MS: based on comparison with WILEY, ADAMS and NIST 08 MS databases

Of all alcohols identified in pine honey samples, nonanol (7.48–28.78%), was found as the most dominant alcohol. Nonanol is an oily, colorless liquid having a citrus odor [31]. Aldehydes were the major aroma compounds in all studied pine honey samples. Aldehydes such as nonanal (21.90–52.57%), decanal (3.62–15.08%) benzaldehyde (0.24–5.01%), octanal (0.39–4.44%), and phenylacetaldehyde (0.23–3.52%) were quantified in all pine honey samples while lilac aldehyde A (3.87%) was only found in pine honey sample of Aydın-Kuşadası (AK1) region. Lilac aldehyde A might be used as a characteristic compound for pine honey sample of AK1 region. Lilac aldehyde B was absent in the studied pine honey samples except for pine honey samples of İzmir and Aydın regions. Terpenes were another most dominant compound in the pine honey samples. Fourteen terpenes were quantified and α-pinene (0.40–10.45%) had the highest level, followed by limonene (0.26–9.16%), borneol (0.87–5.21%), and 1,8-cineole (0.08–4.19%). *α*-Pinene, limonene, borneol, and 1,8-cineole are considered that they have pine, orange, menthol, mint odor properties, respectively. *Cis-β*-ocimene was detected in 7 pine honey samples; thymol in 6 pine honey samples; and bornyl acetate in 2 pine honey samples. Other terpene compounds were common for pine honey samples studied. Two different ketones; namely, 2-nonanone (0.15–2.00%), and 4-oxoisophorone (0.25–6.17%) were identified in all pine honey samples whereas α-isophorone were detected in 8 pine honey samples. Two esters and one acid were found in pine honey samples. Methyl salicylate (0.27–4.82%) was detected in all pine honey samples while isopropyl myristate was identified in 16 pine honey samples; nonanoic acid in 10 pine honey samples.

The volatile compounds present in all studied pine honey samples were octanol, nonanol, 4,4,7-*α*-trimethyl-2,4,5,6,7-*α*-hexahydro-1-benzofuran-2-yl-methanol, benzaldehyde, octanal, phenyl acetaldehyde, nonanal, 2-nonanone, 4-oxoisophorone, methyl salicylate, *α*-pinene, *β*-pinene, limonene, *cis*-linalool oxide, borneol, and *β*-damascenone. safranal, *p*-cymene, 1,8-cineole, and myrtenol were found to be present in most of the samples.

The volatile compounds such as octanol, nonanol, benzaldehyde, octanal, phenylacetaldehyde, nonanal, safranal, lilac aldehyde A, lilac aldehyde B, 2-nonanone, *α*-isophorone, 4-oxoisophorone, methyl salicylate, isopropyl myristate, nonanoic acid, *α*-pinene, *β*-pinene, *cis-β*-ocimene, *p*-cymene, limonene, 1,8-cineole, *cis*-linalool oxide, borneol, myrtenol, eucarvone, *β*-damascenone, thymol, and *trans*-geranyl acetone have been previously reported in the honey aroma [18, 26, 32–41].

To the best of our knowledge,compounds such as 4,4,7-*α*-trimethyl 2,4,5,6,7-*α*-hexahydro-1-benzofuran-2-yl-methanol, and [*E*]-2-octenal, bornyl acetate have not been previously found to be present in honey aroma.

There are few studies on volatile compounds of pine honeys from Turkey. In a recent study, aroma compounds of pine honey samples from two different regions of Turkey identified using solvent-assisted flavor evaporation. In total, 36 compounds were detected and phenylacetaldehyde was found as the most significant aroma-active compound in both studied pine honeys [24]. Aroma compounds of Turkish pine honey samples from Marmaris, Datça, and Fethiye regions using solid phase micro extraction/gas chromatography–mass spectrometry technique were investigated by Bayraktar and Onoğur [26]. The most dominant aroma compounds found to be nonanal, nonanol, decanal, and octanal in the three regions studied. In a previous study, Tananaki et al. [6] were analyzed 44 samples of pine honey which is 22 from Turkey. A total of 77 compounds were identified and the 3-carene was found to be a characteristic compound to Turkish honeys. There are quantitative and qualitative similarities and differences between the results obtained from this study and the literature due to the geographical origin of honey [18, 32].

Chemometric analysis

The volatile compound results were applied to Principal Component Analysis (PCA) to determine whether 23 pine honey samples from 7 different regions can be distinguished. Twenty-two of the 33 volatile compounds were found to be important for the differentiation of studied pine honeys. Thus, 22 compounds were applied to PCA. The results exhibited that the first principal component (PC1) accounted for 25.7% of the total variance while the second principal component (PC2) accounted for 15.6%. Score plot and loading plot graphics were exhibited in Figs. 2 and 3, respectively in terms of PC1 and PC2 in the pine honey samples. It was determined that pine honey samples were divided into 6 groups according to differences and similarities in their volatile compounds when Figs. 1 and 2 evaluated together. While each region clustered among itself, the Manisa region (MT) is clustered together with the Balıkesir region (Bİ, BA, and BH) due to volatile compounds such as *α*-isophorone and *β*-damascenone. It has been determined that the pine honey samples produced from Muğla's regions close to Antalya (MF and MS2) were similar to the pine honey samples produced from Antalya (AK2 and AF). Thus, MF, MS2, AK2, and AF pine honey samples were clustered in the same group. In addition, the Çanakkale region (CG) was clearly separated from those of other regions because of the number of compounds such as α-pinene, benzaldehyde, 4-oxoisophorone, octanal, phenylacetaldehyde, borneol,

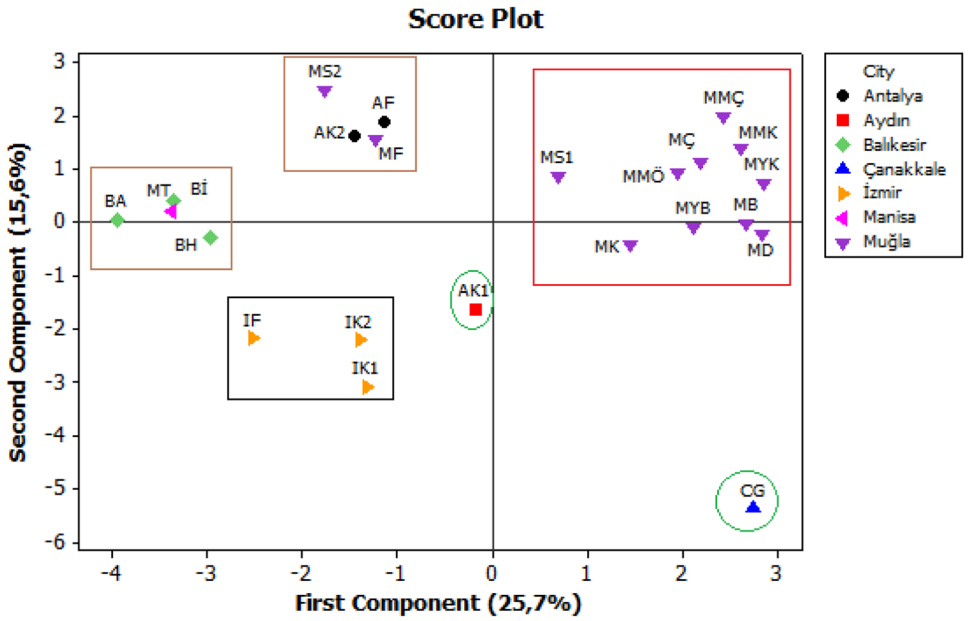


Fig. 2 Score plot graphic in terms of PC1 and PC2 in pine honey samples

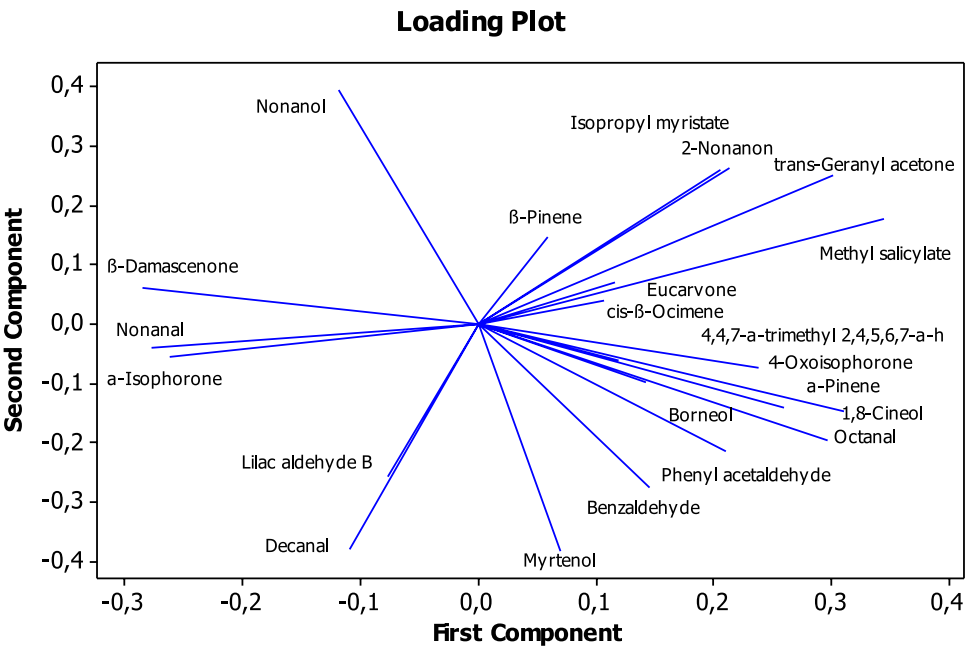


Fig. 3 Loading plot graphic in terms of PC1 and PC2 in pine honey samples

1,8-cineole, and myrtenol. When Fig. 2 is examined, it is determined that the İzmir region is different from the others and the closest region to it is the Aydın region due to the Lilac aldehyde B compound.

Hierarchical cluster analysis (HCA) was used to investigate the similarities of the 7 different regions' pine honey samples and the dendrogram is presented in Fig. 4. The results of 22 volatile compounds in all studied pine honey samples were used in cluster analysis. The dendrogram was prepared using the Euclidean distance and Ward Linkage Method. When Fig. 3 is examined, it was seen that there were 2 main clusters. The first main cluster consists of CG and the second main cluster consist of the other studied pine honey samples. However, it was determined that the pine honey samples of the İzmir region are also separate clusters from the others. In general, the results of Dendrogram analysis were found to be compatible with PCA results.

Conclusions

A detailed study of the volatile composition and chemometric analysis of Turkish pine honeys from different locations was performed. The most dominant compounds were

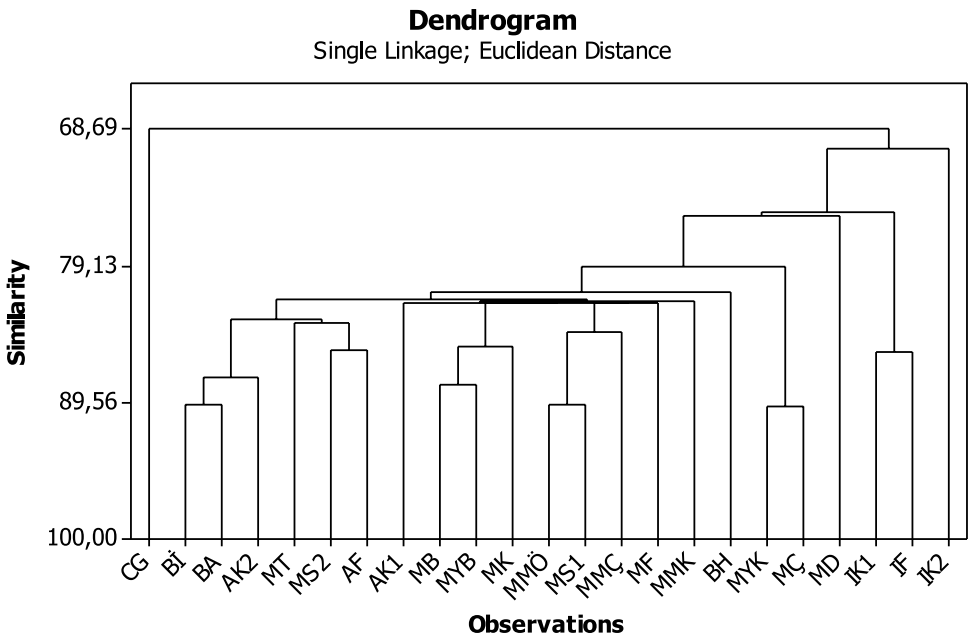


Fig. 4 Dendrogram results obtained by Euclidean distance and Ward Linkage method

aldehydes, alcohols, and terpenes. As a result of the chemometric analysis, we made with 22 volatile compounds; it was determined that pine honey samples according to their place of production were clustered as Northern Aegean, Central Aegean, Southwestern Aegean, and the Mediterranean regions. When the obtained results and chemometric analysis are evaluated together, it has been concluded that nonanal, nonanol, octanol, decanal, phenylacetaldehyde, benzaldehyde, octanal, α-pinene, 4-oxoisophorone, methyl salicylate, isopropyl myristate, limonene, and β-damascenone could be used as marker compounds in the pine honeys produced in Turkey.

In this research, it has been revealed that there are partial differences in the volatile compounds of pine honey according to the production places in Anatolia, where the most pine honey is produced in the world, and pine honeys are clustered according to the qualitative and quantitative differences in the components.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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