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Effect of Seasonal on Strength of Bee and Volatile Compounds in Multi Flora Honey

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Abstract

This study was performed in Basra - Iraq, the Aims of Identify of the seasonal effect on the strength of honeybee colonies and Determination of volatiles compounds in honey by Gas Chromatography-Mass Spectrometry (GC-MS), The results showed significant differences ($p<0.05$) between means , it showed an increase in the area of the Capped brood during the months March, April, May and June, then decreased in July and August, then increase activity during September and October and then decreased in November. the Determination of volatile compounds in spring and autumn honey it was appeared in both types the compounds: Diethyl Phthalate, and Acetic acid, [bis[(trimethylsilyl)oxy]phosphinyl]-, trimethylsilyl ester, and Octadecanoic acid, and Eicosane and Others in Different rates, the volatile Compounds in spring season honey appeared including: Diethyl Phthalate and Acetic acid, [bis[(trimethylsilyl)oxy]phosphinyl]-, trimethylsilyl ester and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- and 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester and Trisiloxane, respectively, while in autumn season honey appeared including: l-(+)- Ascorbic acid 2,6-dihexadecanoate, and Dodecanoic acid, and Diethyl Phthalate, and 6-Octadecenoic acid, and Hexatriacontane, respectively.

Key words: Seasonal change, bee brood, honey , GC-MS ,Volatile Compounds.

Introduction

The length of the honeybees life has been evaluated in All the seasons, it have a apparent bimodal apportionment at the temperature zones, the honeybees live for 30–40 days in spring, while in summer 15–38 days, however in autumn 50–60 days, and for 150–200 days in winter, wherever it has been evaluated the variation of life length between seasons may come of foraging and activity of brood-rearing (Remolina & Hughes. 2008).

The honey bee colonies strength is significantly positively correlated between the amount of brood breeding and The amount of bees in colonies prior nectar overflow correlated with the honey production is highly significantly positively (Gąbka. 2014).

Honey is a natural complex bee product from Multi floral sources may have various tastes and aromas due to several volatile compounds, The geographical and botanical

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of the flora are also based on the methods of extraction for transformation (Jerkovic *et al.,* 2010).So The botanical origin may be determined by a highest concentration of compounds in several types of honey collecting than in others(Castro- Várquez *et al.*, 2006).

Materials and methods

All experiments was performed in Basrah –Iraq in 2019 on 9 colonies of *Apis mellifera* L. bees in hives All queens in hives were one-year old, produced from one reproductive queen, and were naturally intermarry, In the beginning of February until the end of November 6 colonies covering 6 combs. Check each colony included brood , adult bee pests. The Number of brood was estimated of the basis of brood surface area by Squares measuring of 2 cm^2.

Honey samples

Honey produced by two different seasons (spring and Autumn) from honey bee which was in blooming from the beginning in Basrah –Iraq. All The honey samples were producers from *Apis mellifera* the samples were kept in a dark place at 25 °C .

Extraction of volatile organic compounds

The Clevenger SD unit has been used. The boiler was filled with 200 ml of the sample (80 g of honey /100 ml of water). The method was calibrated for 4 hours and the condenser of the unit was cooled for water at 20 °C (Eleftherios *et al.,*2005).

Conditions for GC-MS

The study was carried out using the Shimadzu GC-MS QP2010, The temperature of the injector was 280 °C. Inside the section processing, a solution of honey was injected with a split ratio of 1/60. Column capillary Rtx-5MS95 percent Dimethyl Polysiloxane-5 percent Diphenyl (30 m × 0.25 mm × 0.25 ìm). Career gas used helium at a steady speed of 1,00 mL/min. The oven temperature was as follows: at the first temperature of 60 °C, held for 2 min, then increased for 10 °C/min until 260 °C held for 10 minutes. The MS ionization capacity was 70 eV, the temperatures were as follows: interface 260 °C, source Ions 280 °C. Scanning the mass spectrophotometry from 40-550 (Khan *et al.,* 2017).

Statistical analyses

Statistical analyses were performed and the significance differences between the means of groups were calculated by L.S.D test (Ali *et al.*, 2019).

Results and discussion

The Seasonal change in the levels of capped bee brood:

The Fig 1 showed the change of Capped brood area of honey bees, which was based on various months of the year. the statistical analysis showed significant differences

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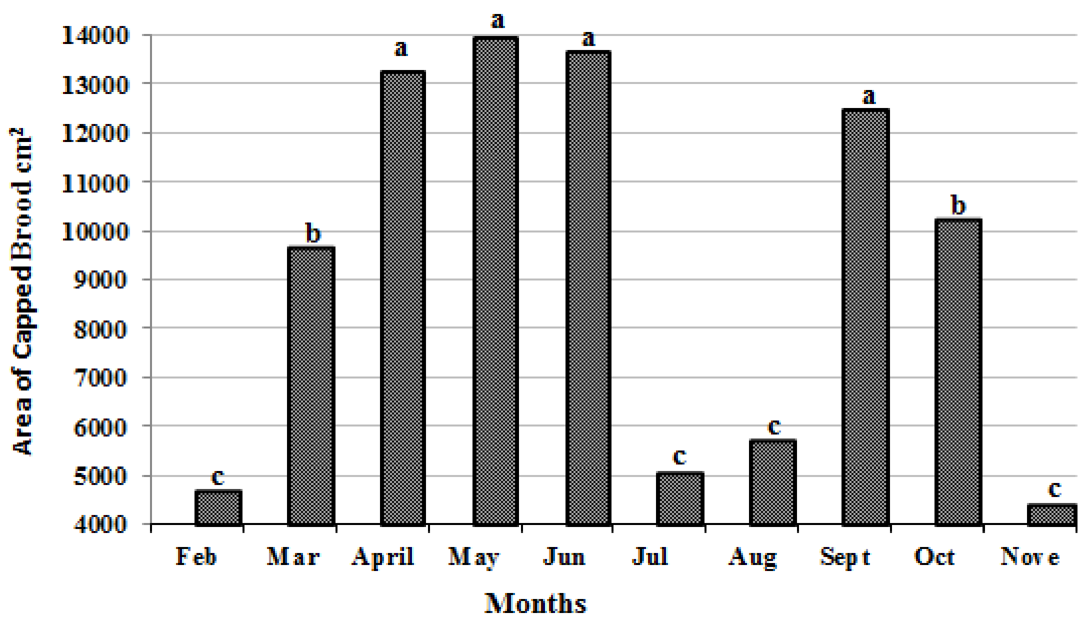
between the means (P< 0.05) The mean of bee brood area in February Values was 4676.5 cm^2 , It increased during March, April, May and June to Values its highest level 9674.4 cm^2, 13245 cm^2 , 13957 cm^2, 13654.6 cm^2 respectively. Then it decreased in July and August, which is the lowest level of the brood Values 5047.3 cm^2 and 5679 cm^2 respectively. then The honeybees reactivated in September and October to 12467.4 cm^2 and 10233 cm^2 respectively. In November the brood area was 4373 cm^2.



***L.S.D: 1525 significant differences (P <0.05) between means**

Fig. 1: Seasonal change in the levels of capped bee brood.

The amount of brood is usually positively correlated with bee colony growth, the exact correlation differing on various sources, In the spring the strong colonies rearward more brood, however the differences are not forever statistically significant, and The observed seasonal variance in dry weight with the highest during April and May partly reflect colony evolution, with higher protein Enabled for larvae breeding in spring (Requier *et al.*, 2015).

The strength of bee colony was proportional with the brood rearing, the strong colonies constantly reared more broods. A larger number of bee workers can care and feed to a larger area of brood (Bhusal *et al.*,2011), So The strength of colony depends on many factors in the spring, but firstly, on climatic conditions, so colony strength in wintering and measures taken during the spring (Jevtić *et al.*,2013).

Gąbka, J.(2014) found the positive correlation between the area of bee brood, honey and the colony strength at April to May, it caused by the reality that a more amuont of worker bees were obtainable to feeding and warming the brood.

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Volatile Compounds in Honey:

The results in Fig 2 and Table 1 show the volatile compounds in honey in the spring season, the diagnosis of volatile compounds in honey by Gas Chromatography Mass Spectrometry (GC-MS) technique, From the figure shown, 50 peaks of volatile compounds are observed which are represented by the peak 14 of Diethyl Phthalate with a similarity of 89% , Followed by the peak 5 of Acetic acid, [bis[(trimethylsilyl)oxy]phosphinyl]-, trimethylsilyl ester with a similarity of 87%, Then the peak 1 was 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- with 96% similarity, Then the peak 31 of the compound 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester with a similarity of 96%, Then the 9th peak, Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]- with the similarity 94%, Then the 40th peak of Cholest-5-en-3-ol, (3.alpha.)-, and the peaks of compounds Benzeneethanamine, N-[(pentafluorophenyl) methylene]-.beta., 3,4 - tris [(trimethylsilyl),17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11, 12,13,14, 15, 16,17-tetradeca, and The compounds of Octadec-9-enoic acid, and other compounds have appeared, including [Dimethyl-(3-trimethylsilanyloxy-propyl)-silanyl]-benzene -, then the compound Octadecanoic acid, and Cyclopropanenonanoic acid, 2-[(2-butylcyclopropyl) methyl]-, methyl ester and other compounds within different Rates.

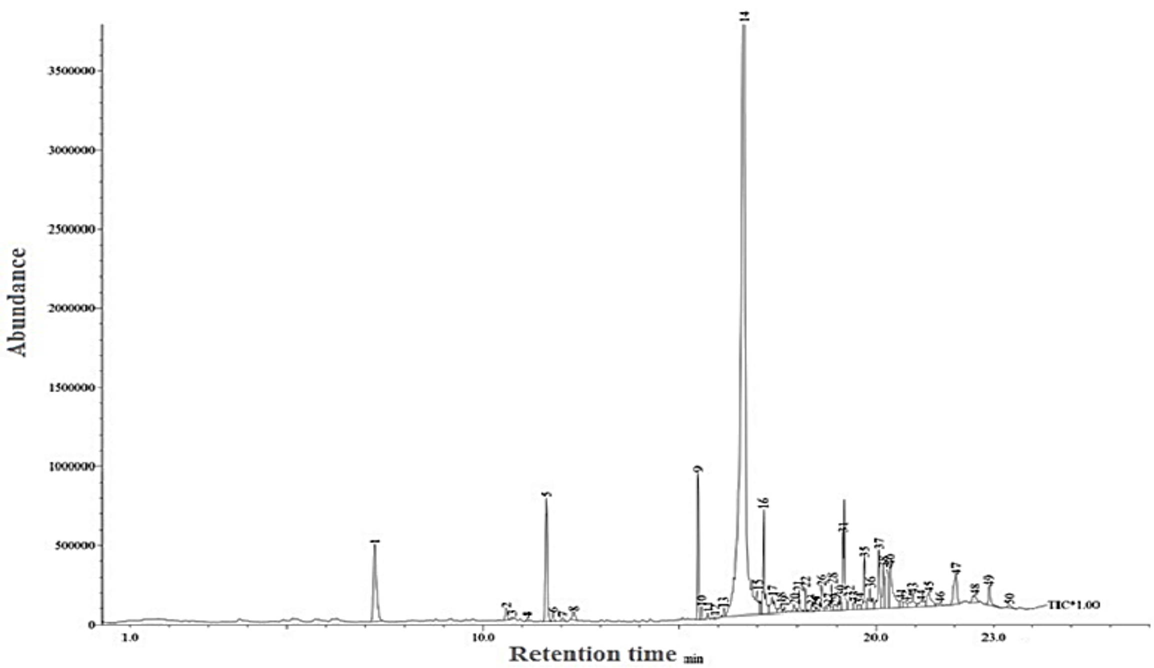

Fig. 2: GC-MS Chromatogram of Spring Season Honey.

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Table 1. Volatile compounds of spring season honey identified by GC-MS

Peak	R.Time	Area%	Name
1	7.251	5.87	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
2	10.579	0.38	Benzeneacetic acid
3	10.730	0.08	Nonanoic acid
4	11.135	0.09	Methoxy-4-vinylphenol
5	11.606	7.07	Acetic acid, [bis[(trimethylsilyl)oxy]phosphinyl]-, trimethylsilyl ester
6	11.779	0.25	Phenol,2-etyl-4methyl-
7	12.039	0.13	Tetradecane, 4-methyl-
8	12.309	0.28	1H-Indene-1,2-diol, 2,3-dihydro-1-methyl-, cis-
9	15.468	4.81	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-
10	15.560	0.20	Dodecane, 2,6,11-trimethyl-
11	15.729	0.15	Heptadecane, 2,6,10,15-tetramethyl-
12	15.914	0.04	1-Octadecanesulphonyl chloride
13	16.120	0.16	1,3,5,7,9-Pentaethyl-1,9-dibutoxypentasiloxane
14	16.658	37.01	Diethyl Phthalate
15	17.002	0.21	Benzophenone
16	17.136	4.58	Benzeneethanamine, N-[(pentafluorophenyl)methylene]-.beta.,3,4-tris[(trimethylsilyl)
17	17.360	1.06	Cyclopropanenonanoic acid, 2-[(2-butylcyclopropyl)methyl]-, methyl ester
18	17.586	0.33	Tetracosane
19	17.684	0.13	Tetradecanal
20	17.935	0.20	2-Propenoic acid, (1-methyl-1,2-ethanediyl)bis[oxy(methyl-2,1-ethanediyl)] ester
21	18.034	0.43	Tetradecanoic acid
22	18.220	0.35	Eicosane
23	18.357	0.17	Octadecanal
24	18.465	0.08	2,6-Octadiene, 1-(1-ethoxyethoxy)-3,7-dimethyl-
25	18.505	0.10	1,2-Epoxynonane
26	18.624	0.70	Phthalic acid, decyl isobutyl ester
27	18.805	0.46	1-(4-Hydroxy-3,5-di-tert.-butylphenyl)-2-methyl-3-morpholinopropan-1-one
28	18.892	0.43	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
29	18.955	0.23	Octadecanoic acid, 3-hydroxy-2-tetradecyl-, methyl ester, (2R,3R)-
30	19.078	0.47	Cyclopentadecanone, 2-hydroxy-
31	19.160	5.03	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester
32	19.331	0.76	Hexadecanoic acid, ethyl ester
33	19.453	0.39	.beta.-D-Glucopyranoside, methyl-4-O-decyl-
34	19.586	0.27	17-Pentatriacontene
35	19.724	3.15	[Dimethyl-(3-trimethylsilanyloxy-propyl)-silanyl]-benzene
36	19.865	0.87	Hexatriacontane
37	20.072	3.79	Octadec-9-enoic acid
38	20.185	3.14	Octadecanoic acid
39	20.276	4.28	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradeca
40	20.365	4.70	Cholest-5-en-3-ol, (3.alpha.)-
41	20.645	0.30	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
42	20.775	0.35	9-Tricosane,(Z)-
43	20.937	0.62	Tetratetracontane
44	21.145	0.62	Oxirane, hexadecyl-
45	21.350	0.99	13-Docosenamide, (Z)-
46	21.645	0.21	Tetratriacontane
47	22.033	3.73	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-
48	22.534	0.50	Tetrapentacontane, 1,54-dibromo-
49	22.878	0.72	1,2-Benzenedicarboxylic acid, diisooctyl ester
50	23.409	0.17	Cholest-5-en-3-ol (3.beta.)-, nonanoate

The Fig 3 and Table 2 show the volatile compounds in honey in the autumn season the diagnosis it through by the (GC-MS) technique, From the figure shown, 58 peaks of volatile compounds are observed which are represented by the peak 36 of l-(+)-

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Ascorbic acid 2,6-dihexadecanoate, with a similarity of 89% , Then the peak 17 was Dodecanoic acid with the similarity 96% , and the 18th peak, which was Diethyl Phthalate with similarity 94% ,then the 41th peak, which was 6-Octadecenoic acid, (Z)- with similarity 94%, and the peak 40 was Hexatriacontane with the similarity 96% ,and the peak 52 was 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- with similarity 94% ,then the 47th peak which was compounds Tetratetracontane, then 2,5-Furandicarboxaldehyde, and Tetradecanoic acid,and Tetrapentacontane, 1,54-dibromo-, and Octadecanoic acid, and Cholest-5-en-3-ol, (3.alpha.)-Respectively and other compounds within a different rates.

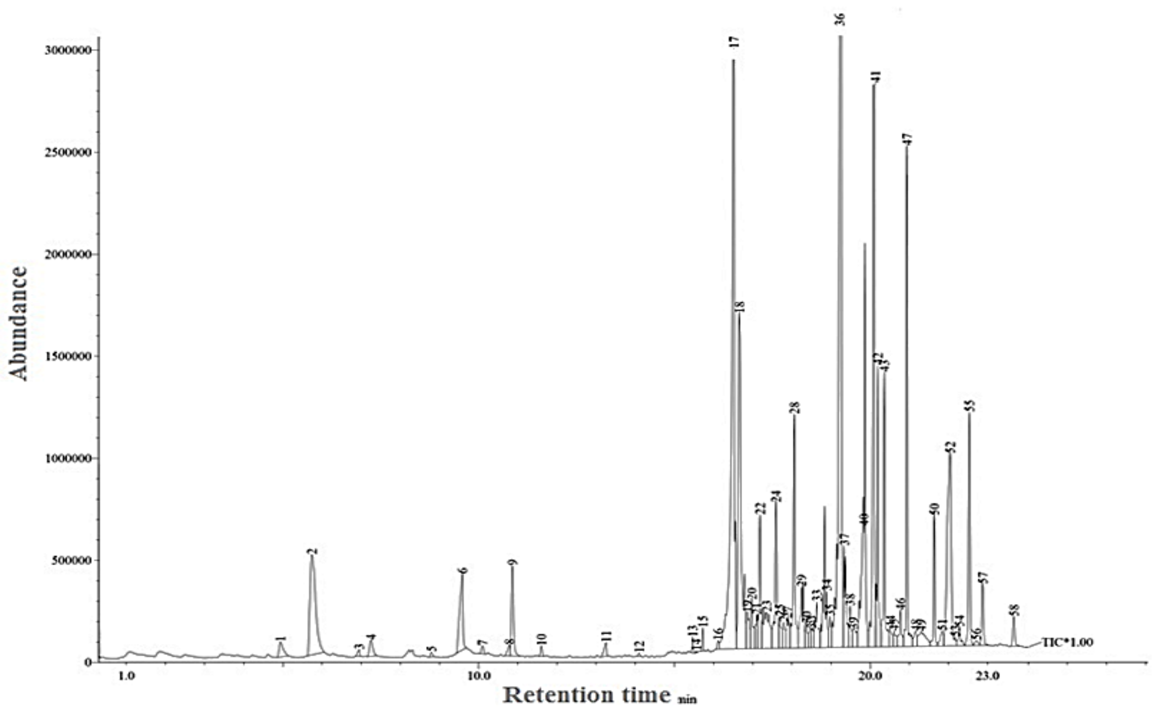

Fig. 3: GC-MS chromatography of the autumn season of honey.

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Table 2. Volatile Compounds in Autumn Season Honey Identified by GC-MS

Peak	R.Time	Area%	Volatile compound
1	4.934	0.42	Benzeneacetaldehyde
2	5.738	3.93	2,5-Furandicarboxaldehyde
3	6.946	0.10	Hexanoic acid, 2-ethyl-
4	7.249	0.37	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
5	8.793	0.06	Decanal
6	9.582	1.68	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
7	10.099	0.12	Benzaldehyde, 4-methoxy-
8	10.785	0.18	Nonanoic acid
9	10.864	1.37	Benzene, 1-methoxy-4-(1-propenyl)-
10	11.601	0.12	Acetic acid, [bis[(trimethylsilyl)oxy]phosphinyl]-, trimethylsilyl ester
11	13.253	0.23	n-Decanoic acid
12	14.098	0.04	Tetradecanal
13	15.464	0.12	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-
14	15.556	0.01	Dodecane, 2,6,11-trimethyl-
15	15.724	0.15	Heptadecane, 2,6,10,15-tetramethyl-
16	16.115	0.11	1,3,5,7,9-Pentaethyl-1,9-dibutoxypentasiloxane
17	16.516	13.57	Dodecanoic acid
18	16.661	6.90	Diethyl Phthalate
19	16.859	0.74	Epicedrol
20	16.973	0.70	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)-
21	17.102	0.44	2,6-Difluoro-3-methylbenzoic acid, dodecyl ester
22	17.186	1.50	Propylamine, N-[9-borabicyclo[3.3.1]non-9-yl]-
23	17.358	1.76	1,2-Benzenedicarboxylic acid, ditridecyl ester
24	17.592	2.18	Heptadecane
25	17.700	0.50	Tridecanal
26	17.790	0.43	Acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester
27	17.882	0.56	Tetradecanal
28	18.064	2.96	Tetradecanoic acid
29	18.222	1.50	Eicosane
30	18.370	0.35	15-Octadecenal
31	18.464	0.30	2,6-Octadiene, 1-(1-ethoxyethoxy)-3,7-dimethyl-
32	18.500	0.30	2-Decanone, 5,9-dimethyl-
33	18.640	0.95	Pentadecanoic acid
34	18.890	2.07	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
35	18.975	0.43	Hexadecanoic acid, methyl ester
36	19.255	15.83	l-(+)-Ascorbic acid 2,6-dihexadecanoate
37	19.336	1.58	Hexadecanoic acid, ethyl ester
38	19.485	0.58	i-Propyl 14-methyl-pentadecanoate
39	19.570	0.48	Sulfurous acid, octadecyl 2-propyl ester
40	19.840	5.45	Hexatriacontane
41	20.097	6.69	6-Octadecenoic acid, (Z)-
42	20.197	2.49	Octadecanoic acid
43	20.361	2.38	Cholest-5-en-3-ol, (3.alpha.)-
44	20.520	0.34	Oxirane, hexadecyl-
45	20.610	0.28	Tributyl acetylcitrate
46	20.776	0.57	9-Tricosene, (Z)-
47	20.940	4.08	Tetratetracontane
48	21.162	0.32	Pentadecane, 8-hexyl-
49	21.302	0.76	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-
50	21.639	1.22	Tetratriacontane
51	21.837	0.35	Tetrapentacontane, 1,54-dibromo-
52	22.043	5.29	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-
53	22.170	0.02	Pentadecane,8,8-diheptyl-
54	22.279	0.32	Z-12-Pentacosene

55	22.536	2.51	Tetrapentacontane, 1,54-dibromo-
56	22.703	0.12	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
57	22.874	0.74	1,2-Benzenedicarboxylic acid, diisooctyl ester
58	23.670	0.42	Pentacosane.13-undecyl-

The main factors responsible for aroma are volatile organic compounds with many other factors like the flavor contribute, taste and physical, The volatile organic compounds can be produced from the nectar origin, from the conversion of flora compounds by the digestion process of the bees gut, from the honey handling, processing, during store from environmental, and microbial contamination (Jerkovic *et al.*, 2009).

The variations between types of honey was dependent on differences on geographical origins, plant varieties and beekeeping practices. then, the botanical origin of honey must be determined Depending on plants metabolites including: terpenes, benzene, norisoprenoids, and its derivatives (Castro- Várquez *et al.,* 2006).

The chemical groups in the volatile compounds of honey belong included: aldehyde, hydrocarbon, alcohol, ketone, ester, furan, acid, pyran and benzene, ,terpenes and its compounds, norisoprenoids and cyclic compounds and sulfuric compounds (Barra *et al.*,2010).

Conclusions

The strength of honey bees varies according to the seasons of the year, as well as the honey components of volatile compounds. GC-MS is a possible, reliable method in screening of multi flora honeys for identification volatile compounds, This method can be diagnose the identity of honey quality by different volatile compounds.

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