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DETERMINATION OF VOLATILE ORGANIC COMPOUNDS IN LOCAL HONEY BY GAS CHROMATOGRAPHY–MASS SPECTROMETER

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ABSTRACT

In Saudi Arabia, there are many types of honey which are produced from different flora. They are called Seder, Talh and Zohor. Seder honey is preferable by most of the consumers because of its delicious taste and pleasant odor. The taste and odor of honey are attributed to the volatile organic compounds present as minor constituents.

This paper will present results of three types of honey namely: seder, talh and zohor. The extraction of the organic compounds from honey samples were performed using four different methods. Separation and identification of these compounds has been undertaken using Gas chromatography-Mass spectrometer.

INTRODUCTION

Honey is considered as one of many naturally produced energetic foods. It contains different valuable nutrients which include sugars, minerals, vitamins, enzymes, flavoring organic compounds and other materials.[1]

The composition of honey has been studied extensively, and most of these studies have been directed towards the analysis of non volatile components such as sugars and moisture.[2] Hausler and Montage (1990) were found some honey contain numerous mono- and sesquiterpenoid compounds[3]. It has been indicated that most types of honey in Saudi Arabia are mono-floral [4]. These are Seder (*Zizphus spina* Christi), Salam (*Acacia ehrenbergiana* Hayne), Talh (*Acacia albida* ***Del***), and

Zohor (*Acacia etbaica* Schweinf) which distributed in southern and western regions. Carlo, Flavo and Carllota (1983) analyzed multi floral honeys produced in different years in order to identify the components responsible for the flavor[5], fifty two volatile components were identified by GC/MS. Thirty New Zealand compounds had been examined by Tan, Wilkins, and Reid, (1986)[6], most of them were described as monofloral type. Bonga, Giumanini, and Gliozzi (1986)[7] found the n-hexane extracts of chestnut honey had hydrocarbons more than other class of compounds; twenty six n-alkanes (C_{10} –C_{37}) and ten alkenes (C_{23} –C_{27}) were identified. Alkanes consisted nearly 36% of the total hydrocarbons, and alkenes 64%. Steeg and Montage[8] (1987) studied extensively the aromatic carboxylic acids in honey; the free acids were separated and detected after converting them into trimethylsilane (TMS) derivatives with triflorobistrimethyl silylacetamide. Giorgio and Angelo (1986) have conducted a study on the volatile fraction of chestnut honey.[9] New Zealand leather honey was studied and analyzed by Tan, Wilkins, Ried, and Molen (1986) [10]. Also other type of Newzeland honey was studied by Tan, Holland and Meghie (1990) [11], aromatic acids, phenols, aliphatic acids, diacids, and degraded caroteniods were detected. Multi dimensional analysis have been used to determine 1,2-ketols from honey [12] by Mosandle, et.al (1991).The volatile compounds of eighty four unifloral honeys have been determined by Bouseta, Sonia and Jean [13] (1992). Maojuan, Donald, and Peter (1993) developed a new method to determine L-menthol in honey at level as low as 0.1ppm.[14] The flavanoids were determined in honey by Berahia, Cerrati and Sabatier [15] (1993), the patterns of honey could be used as a characteristic in determination of floral origin. The volatile and semi volatile organic compounds in honey were analyzed by Sanford and John (1994).[16] Singh and Bath (1997)[17] investigated the chemical composition, flow behavior indices and overall acceptability of the most popular types of honey produced in India. They also studied[18] (1999) the microwave heating effect on the *Helianthus annus* and *Eucalyptus lanceolatus* honey for the hydroxymethylfurfural (HMF) formation. Amine, Safwat, and El-Iraki (1999) studied the quality criteria of black honey produced in Egypt.[19]. Rashed and Soltan (2004) studied the chemical composition for trace and major elements of bee honey [20]. The chemical studies of Saudi honey are scarce. Messallam, and Shaarawy (1987)[21] studied non volatile compounds such as carbohydrate, protein, and enzymes in the honey. Also Messallam, and Shaarawy (1987)[22] studied the chemical constituents including water, sugar, insoluble solid, acidity and diastase activity.

In this paper the volatile organic compounds of some local honey produced in Saudi Arabia were determined by gas chromatography – mass spectrometer.

EXPERIMENTAL

Honey samples

Three types of honey produced from different areas of the southern regions of Saudi Arabia were analyzed. *Zizphus spina* (Seder), *Acacia albida* (Talh), and *Acacia etbaica* (Zohor).

Procedural

The procedural blank is extracted as the sample of interest as a check on concentration during sample preparation. A procedural blank should be extracted with each set of samples and accompany that set of samples through all analytical stages. In the study, four different blank solutions have been done for various methods of extraction under the same analytical conditions of each method.

Reagents

Quinoline, phenylacetaldehyde, p-anisaldehyde, benzaldehyde, and linalool standards were obtained from (Fluka). Deionized water was obtained from a Millipore Milli-Q water purification system (Bedford, MA, U.S.A) was used for all solutions. Anhydrous sodium sulfate (Fluka) was used as a drying agent. Dichlomethane was HPLC-grade (Fluka)

Steam Distillation / Solvent Extraction

20-30 g of crude honey was weighed and transferred into 250 mL round bottom flask. 100mL deionized water was added and then the flask was shaken vigorously until the crude honey was completely dissolved. 50mL of dichloromethane (DCM) was added from the top of the steam distillation /extraction apparatus and the apparatus was placed on the top of the round bottom flask. The solution was heated using a heating mantle (at 110° C). The boiling temperature was controlled using a temperature thermostat. The distillate was collected and another 20mL of DCM was added from the top of the apparatus and then collected. The combined solution was transferred to a separatory funnel (200mL) and was shaken vigorously for 10 minutes. The mixture was left to settle down for 30 minutes. The DCM layer was transferred into 500mL flask. The aqueous layer was then extracted with 10mL and 20mL DCM respectively. All extracts were combined and dried over anhydrous sodium sulfate. The extracts were concentrated to about 2ml under vacuum on a rotary evaporator at 35° C then completely evaporated under nitrogen and kept in the refrigerator at 15° C till the analysis.

Purge & Trap

In this method 1.69 g of one type of Seder honey produced from Asser region was introduced into a 10 mL test tube and heated to 60°C. The honey sample was sparged with high purity helium at 40 mL / min. for one hour. Volatile analytes were extracted and carried out to a desorption tube packed with desorption material, normally is 2,6-Diphenylene oxide polymer (Tenax, 60-80 mesh). The tube with the sample was then attached to a thermal desorption system and injected into the GC injection port at desorption temperature of 240°C for 9 minutes.

INSTRUMENTATION

GC/MS/Quadruple

All GC/MS analysis was done on Shimadzu QP5000 gas chromatograph /Mass spectrometer (Shimadzu Corporation, Kyoto, Japan). The gas chromatograph (GC Shimadzu 17A) was equipped with a fused silica capillary column (30m × 0.25mm i.d, 0.25um film thickness, DB-5 (5%phenyl silicon, 95%methyl silicon) (J&W) Scientific, Rancho Cordoba, CA, USA). The end of the GC column was introduced into electron impact (EI) source of quadrupole mass spectrometer through the interface which has temperature of 250°C. Samples were analyzed in full data acquisition (scan) mode. Data acquisition was carried out using a class 5000 data system. All analysis was performed using split injection mode with split ratio of 1:25, and helium as a carrier gas with flow rate of 1.8mL / min.

GC/MS/TD/Ion Trap

Varian GC-MS (GC-STAR 3400Cx, MS-SATURN 3) has been also used in the analysis of one type of honey (Seder honey). This system was equipped with purge & trap (model 30) system and thermal desorption (TD, model 890) unit. The GC was equipped with a fused silica capillary column (60 m x 0.25mm i.d , 1.5um film thickness , DB-5(J&W Scientific)). The end of the GC column was introduced into the electron impact (EI) source of ion trap mass analyzer.

RESULTS AND DISCUSSION

The identification of the mass spectra in both systems was achieved with the aid of NIST library, and comparison with published mass spectra. Retention times and comparison with authentic samples was applied whenever possible. In addition, the identification of the isolated organic compounds was done by interpretation of their mass spectra fragmentation patterns. The peaks eluted at short retention times were mostly volatile oxygenated compounds (e.g. aldehydes, ketones,etc.) while those eluted at long retention times were semi volatile compounds (e.g. long chain hydrocarbons and fatty acids). Compounds isolated by steam distillation/solvent extraction were found to belong to a number of organic

classes namely; heterocyclic compounds (furan derivatives, quinoline, and pyrrol), oxygenated compounds (aldehydes, ketones, esters, alcohols, and carboxylic acids), and hydrocarbons (cyclic, aromatic and aliphatic).Twenty five organic compounds (Table 1) have been extracted from seder honey using steam distillation/solvent extraction method, five of which have not been reported previously in honey. Table 2 shows the volatile organic compounds in seder honey identified by Purge and Trap instrument. Tables 3 shows the six components are common in both techniques. Table 4 and 5 summarized the volatile organic compound in talh and zohor honey.

Organic compounds

It is a well established that the types of organic compounds common to given samples of honey are dependent upon the type of flora present in region as well as enzymatic processes in bees [1].

The heterocyclic compounds in this study were furan derivatives including acetylfuran, methyl-2-furoate, benzofuran, 5-hydroxymethylfurfural, and 5-methyl furfural, quinoline and 2-acetylpyrrol. The presence of furan derivatives in honey extracts has been ascribed to sugar degradation [14] during long storage, heating process and /or distillation process. Such compounds are undesirable in large traces in honey [14]. 5-methyl-2-furancarboxaldehyde was detected in seder honey (produced from Shamran and Asser region) and Talh honey with low relative intensity. Using purge & trap technique, this compound was detected in seder honey with high relative intensity. The characteristic fragments of this compound were observed at m/z 53 as a base peak and at m/z 110 as a molecular ion M^+. 2,3-dihydrobenzofuran was only detected in Talh honey with very low abundance. The mass spectrum of this compound shows the main peak at m/z 91 due to the loss of (CHO) and M^+ at m/z 120.

The effect of excessive temperature of sugar in honey can be recognized by the present of the 5-hydroxymethylfurfural (HMF) due to a break down of sugar [23]. A trace of HMF is always present naturally in honey, but this rarely exceeds 10 mg/kg in fresh material. This quantity can exceed 30-40 mg/kg through the adverse and very long storage or over heating. A content of more than 150 mg/kg is taken to indicate adulteration with invert sugar [23]. The HMF was not detected in seder (produced from Asser region), and zohor, and this reflects the quality of these types. However, HMF was detected in some types of Seder honey (i.e. Balgarn, Shamran) with different relative intensity. The quality of this type of honey is not as high as Balgarn honey. This may be due to long bad storage conditions of honey. Wootton, Edwards, Faraj, and Williams (1978) found that the storage of honey increases the amount of HMF [24]. This compound was not detected in seder honey (produced in Asser region) by using purge and trap technique and therefore, the effect of temperature can be recognized well.

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ÖZET: Suudi Arabistan'da farklı floradan üretilen çok farklı ballar vardır. Bunlar Seder, Talh ve Zohor olarak adlandırılır. Seder balı lezzeti ve hoş kokusu nedeniyle tüketicilerin çoğu tarafından tercih edilir. Balın tadı ve kokusu az bulunan bileşenlerden uçucu organik bileşiklerden kaynaklanır. Bu yayında, seder, Talh ve Zohor adlı üç tip balla ilgili sonuçlar sunulmuştur. Bal numunelerinden organic bileşiklerin ekstraksiyonu dört farklı yöntem kullanılarak gerçekleştirilmiştir. Bu bileşiklerin ayrılması ve yapılarının aydınlatılması gaz kromatoğrafikütle spektrometresi kullanılarak yürütülmüştür.

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Table 1 Organic compounds identified in seder honey using steam distillation / solvent extraction

No	M.W	Name of the compound
1	112	3-Hexen-2,5 dione(Diacetyl ethylen)
2	126	5-Hydroxy methylfurfaral
3	194	Dimethyl phathalate
4	144	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one
5	126	Methyl 2-furoate
6	278	Diisobutyl phthalate
7	109	1-(1H-pyrrol-2yl) ethanone
8	98	2-Methylcyclopentanone
9	120	2,3-Dihydrobenzofuran
10	154	3,7-Dimethyl-1,6-octadien-3-ol (linalool)
11	154	3-Cyclohexene-1-menthol(α-terpineol)
12	106	Benzaldehyde
13	129	Quinoline
14	110	5-Methyl-2-furfural
15	135	3-Amino acetophenone
16	110	2-Acetylfuran
17	152	2,6,6-Trimethyl-2-cyclohexene-1,4 dione
18	122	2-Phenylethylalcohol
19	222	Farnesol
20	138	3,5,5-Trimethyl-2-cyclohexene-1-1one (Isophoron)
21	152	3,7- Dimethyl-1,5,7-octatrien-3-ol (Hotrieneol)
22	196	Methyl 3,5-dimethoxy benzoate
23	170	Linalol oxide
24	120	Phenylactaldehyde
25	136	p-Anisaldehyde(4-methoxy benzaldehyde)

Table 2 Organic compounds identified in seder honey by purge & trap technique

No	M.W	Name of the compound
1	100	Methyl isobutyl ketone
2	88	Butanoic acid
3	92	Toluene
4	137	Carbamic acid, phenyl ester
5	102	3-Methyl Butanoic acid
6	102	2- Methyl Butanoic acid
7	100	Dihydro-2-methyl 3(2H)-furanone
8	102	Pentanoic acid
9	96	2-Furancarboxaldehyde
10	98	2-Furanmethanol
11	118	2-Butoxy ethanol
12	96	4-Cyclopenten-1,3-dione
13	116	Hexanoic acid
14	110	1-(2-furanyl)ethanone
15	114	2,5-Hexanedione
16	126	6-Methyl-5-hepten-2-one
17	112	5-Methyl-5-hexene-2-one
18	110	5-Methyl-2-Furancarboxaldehyde
19	124	2-Acetyl-5-methylfuran
20	142	2-Nonen-1-ol
21	120	Benzenacetaldehyde
22	152	3,7 dimethyl 1,5,7-octatrien-3-ol (hotrineol)
23	109	1-(1H-pyrrol-2-yl)ethanon
24	124	2-Methoxy phenol
25	144	Octanoic acid

26	124	6-Methyl-2-purazinylmethanol
27	138	3,5,5-Trimethyl-2-Cyclohexene-1-one
28	198	2-Tridecen-1-ol
29	129	Isoquinoline

Table 3 Common identified organic compounds in both techniques

No	M.W	Name of the compound
1	110	5-methyl-2-Furancarboxaldehyde
2	120	Benzenacetaldehyde
3	152	3,7-dimethyl 1,5,7-octatrien-3-ol, (hotrineol)
4	109	1-(1H-pyrrol-2-yl) Ethanone
5	138	3,5,5-trimethyl 2-Cyclohexane-1-one
6	129	Isoquinoline

Table 4 Organic compounds identified in Talh honey

No	M.W	Name of the compound
1	110	2-Acetylfuran
2	98	2-Methylcyclopentanone
3	112	1,2Diactylethylene
4	96	Furancarboxaldehyde
5	121	Phenylacetaldehyde
6	109	1-(1H-pyrrol-2-yl)ethanon
7	126	Methyl2- furoate
8	170	Linalool oxide
9	154	Linalool
10	144	Pyran

11	154	alpha-Terpineol
12	136	2,3Dihyrobenzofuran
13	126	5-Hydroxymethylfurfural
14	196	Methyl-3,5-dimethoxy benzoate
15	278	Diisobutylphthalate

Table 5 Organic compounds identified in Zohor honey

No	M.W	Name of the compound
1	121	Phenylacetaldehyde
2	170	cis- Linaloloxide
3	170	trans-Linaloloxide
4	152	Hotrineol
5	122	Phenylethylalcohol
6	138	Isophoron
7	136	p-Anisaldehyde
8	278	Diisobutylphthalate