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Comparative Study of 11 Honey Samples Sold in Riyadh - Saudi Arabia

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دراسة مقارنه لـ 11عينة عسل تباع في مدينة الرياض – المملكة العربية السعودية

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11عينة عسل تم جمعها من عدة محلات تجارية في مدينة الرياض– المملكة العربية السعودية , تهدف هذه الدراسة لمعرفة عما اذا كانت هذه العينات طبيعية ام مغشوشة بالاضافة الى سلامتها للاستخدام الادمي خصوصاً من حيث وجود المواد السامة أو غيرها , حيث تم فحص هذه العينات كيميائياً وبكتيرياً.

مستوى العناصر المعدنية (الحديد, الفسفور, الكالسيوم, البوتاسيم , المغنسيوم) والمعادن السامة (الرصاص, الزئبق, الزرنيخ, الانتيمون, الثاليوم) تم فحصها بجهاز ICP-MS .

تم قياس مستوى الكربوهيدرات مثل الفركتوز والجلوكوز والسكروز والمالتوز والنسبة بين الجلوكوز والفركتوز بواسطة جهاز HPLC. العينات ايضا تم فحصها من حيث وجود السيانيد والزرنيخ من عدمه , وكذلك وجود حبوب اللقاح , وايضا معدل الـ Ph.

كذلك تم فحص العينات كيميائيا لمعرفة المواد العضوية الموجودة في العينات وذلك بجهاز GS-MS , وبينت الدراسة وجود المواد التالية في العينات المفحوصة : السيرنجالدهايد , مينثوفيوران , ترايميثوكسي بنزايل ميثايل ايثر , مسك امبريتي , مشتقات الفينول , هياسينثين , دايهايدروميثايل جاسمونيت , مالتوكسازاين , مشتقات الاسبيدوسبيرماين , فيرسالايد , مشتقات البايران–4-ون

من نتائج الدراسة وجد ان ست عينات من 11 عينة صالحة لاستخدام الانسان, وان ثلاث عينات غير صالحة نظرا لاحتوئها على بعض المواد السامة . بينما كان هناك عينتان لم يعتد بنتائجهما نظرا لانها كانتا مغشوشتين بالسكر.

Key words: *Carbohydrate profile, GC-MS, Honey, Human use, Toxic metals.*

ABSTRACT

11 honey various samples commercially available in Saudi Arabia, were subjected to chemical, toxicological and bacteriological tests. The samples were checked for As and CN, presence of pollens, and for bacteria contamination. The carbohydrate profile was analyzed with HPLC. Fe, P, Ca, K, and Mg were measured with colorimetry. Toxic metals, As, Pb, Sb, Tl, and Hg were determined with ICP-MS. The samples were investigated by GC-MS. The investigation revealed the presence of syringaldehyde, menthofuran, trimethoxybenzyl methyl ether, musk ambrette, phenylacetaldehyde, dihydrojasmonate, maltoxazine, aspidospermine derivative, versalide, and pyran-4-derivative. 6 of the honey samples out of 11 were found to be suitable for human use. 3 samples were not recommended for human use due to the presence of toxic fragrance. 2 samples were adulterated with sucrose (sugar) and therefore were not fully authentic.

Introduction

Many parts of the world produce honey, it is an important economic factor. It has a nutritional value and is also used as a sweetening agent. Honey has been used as a medicine throughout the ages and in recent time has been used by medical profession for treatment of wounds (Hamzaoglu *et al.*, 2000, Molan, 2001, Cooper, *et al.*, 2001, Moor *et al.*, 2001, Armon, 1980, Nadyisaba *et al.*, 1993, Alwaili and Saloom, 1999, Oryan and Zaker 1998), burns (Subrahmanyam, 1991, Molan, 2001, Cooper *et al.*, 2002), leg ulcer (Ankra-Badu, 1992, Alcaraz and Kelly, 2002, Oluwatosin *et al.*, 2000), pressure sores (Hutton, 1966), infant botulism (Cox and Hinkle, 2002, Tanzi and Gabay, 2002), integumental disease in invertebrate (Williams, 2001), chronic hypersensitivity pneumonitis (Fujiwara *et al.*, 1999), chronic bronchitis and bronchial asthma (Bacvarov, 1970), chronic tonsillitis (Kachnyi, 1980), Fournier's gangrene (Eke, 2000) fatal desquamative interstitial pneumonia (Wigger *et al.*, 1977), pneumocystis carinii infection (Weir *et al.*, 1986), chronic seborrheic dermatitis and dandruff (Al-Walil, 2001) and Chronic inflammatory polyarthritis (Camara, 1999). Honey has been reported to have antibacterial and antifungal activity both *in vitro* and *in vivo* (Jeddar *et al.*, 1985, Efems, 1992, Steinberg *et al.*, 1996, Cooper and Molan, 1999, Tichy and Novak, 2000, Ceyhan and Ugar, 2001). Pure commercial honey is composed of approximately 40% glucose, 40% fructose and 20% water. It contains the amino acids glycine, proline, and methionine.

The vitamins biotin, nicotinic acid, folic acid, pantothenic acid, pyridoxin, and thiamine. The enzymes diastase invertase, glucose oxidase, and catalase. And the minerals potassium, iron, magnesium, copper and calcium (Bergman,1983).

The purpose of this study was to investigate the safety of various types of honeys which are sold in Saudi Arabia.

Materials and Methods

1 . Honey samples

11 honey samples were bought from local markets in Riyadh – Saudi Arabia. The description of the samples is given in Table 1 .

Table 1
Color and opaqueness of the 11 honey samples .

Sample	Color	Opaqueness
1	Light-amber	Opaque
2	Medium-amber	Opaque
3	Yellow	Opaque
4	Light-amber	Opaque
5	Dark-amber	Clear
6	Dark-amber	Opaque
7	Very Dark-amber	Clear
8	Medium-amber	Opaque
9	Creamy-white	Opaque
10	Medium-amber	Clear
11	Dark-amber	Clear

2 . Short profile trace – element by ICP/MS

The different honey samples were analyzed for short profile trace elements including, arsenic, lead, antimony, thallium, and mercury using Elan Inductively Coupled Plasma Mass Spectrometry Technique using an Elan 6100 Perkin Elmer instrument.

The honey samples were digested by Microwave Teflon vessel into concentrated metal grade nitric acid. The digested samples were then diluted with 1% (v/v) nitric acid spiked with 10ppb rhodium and 2ppb bismuth as internal standards.

The sample solutions together with blanks, standards, and quality controls were then aspirated directly into Elan ICP/MS technique for the determination of these trace elements at part per billion levels.

3 . Sugars in by HPLC

Fructose, glucose, sucrose, and maltose in honey were extracted with Type 1 water and analyzed with HPLC (Waters Breeze) using an LC-NH2 column. Acetonitrile/water was used as a mobile phase and aspectrophotometric detector set at 196 nm.

4 . GC/MS examination

Honey samples, were extracted with dichloromethane/isopropanal, the extract was evaporated to dryness, and reconstituted in 200µL of the same solvent. Samples were analyzed by GC/MS (Thermo finnigan, DSQ), and investigated for toxic compounds using a software library search option.

Results And Discussion

Examined samples of honey were not contaminated with toxic metals arsenic, lead, antimony, thallium, or mercury (Table 2). The levels of Fe, Ca, P and Mg were generally in the reference range (Table 3). The level of K was higher than the reference range in samples 5, 7 and 11. However this finding is not of toxicological relevance. The levels of fructose, glucose, sucrose, maltose, and fructose/glucose are given in table (4). There was no CN in the samples, and there was no contamination with the microorganisms. The pH values were generally in the reference range (Table 5).

Table 2
The results of examination for As, Pb, Sb, Tl and Hg (in ppb = µg/kg)

Sample	As	Pb	Sb	Tl	Hg
1	nd	7	nd	nd	Nd
2	nd	16	nd	nd	Nd
3	nd	17	nd	nd	Nd
4	nd	13	nd	nd	Nd
5	nd	34	nd	nd	Nd
6	nd	7	nd	nd	Nd
7	nd	1	nd	nd	Nd
8	nd	6	nd	nd	Nd
9	nd	20	1	nd	8
10	nd	13	nd	nd	Nd
11	nd	nd	nd	nd	Nd

Table 3
The results of examination for Fe, P, Ca, K and Mg (in mg/100 g) .

Sample	Fe	P	Ca	K	Mg
1	0.25	1.7	1.7	20.6	0.9
2	0.61	4.7	5.6	59.3	3.3
3	0.33	1.3	2.0	12.1	0.5
4	0.13	2.2	2.9	37.4	1.0
5	0.87	3.4	5.7	144.4	2.9
6	1.08	5.9	7.8	75.1	3.7
7	1.11	11.6	5.9	218.8	7.4
8	0.37	3.4	5.3	26.8	1.5
9	1.73	4.6	2.0	35.9	1.7
10	1.23	8.7	4.4	26.4	4.5
11	0.30	2.9	5.1	252.7	3.1
Mean*	0.30	6.0	5.0	51	3.0

***Geigy Scientific Tables Vol. 1**

Table 4
The results of examination on carbohydrate profile.

Sample	Fructose %	Glucose %	Sucrose %	Maltose %	Fruc/Gluc Ratio	Moisture %	Specific gravity
1	32.7	29.8	10.9	0.0	1.10	13.4	1.421
2	31.3	31.6	0.2	2.1	0.99	14.4	1.428
3	31.5	27.4	4.4	0.0	1.15	16.0	1.396
4	37.3	32.0	0.0	0.0	1.16	15.8	1.386
5	32.6	28.5	0.0	0.0	1.14	18.2	1.404
6	34.4	29.8	0.5	0.0	1.16	17.6	1.436
7	30.0	25.6	17.2	3.6	1.17	13.4	1.427
8	31.0	24.3	3.5	0.0	1.28	13.2	1.413
9	32.8	19.0	0.0	0.0	1.73	18.8	1.404
10	30.1	16.5	0.0	0.0	1.83	14.4	1.438
11	35.2	25.7	0.0	0.0	1.37	16.6	1.407
Range	30.9-44.2	22.9-40.7	0.7-6	0.05 mean	0.76-1.68	17.6 mean	1.4 mean

Table 5
The of examination on pH , As (preliminary test) , CN , presence of pollens and bacterial contamination .

Sample	pH	As	CN	presence of pollens	Microbiology
1	4.30	neg	neg	present	acceptable
2	3.98	neg	neg	present	acceptable
3	3.66	neg	neg	present	acceptable
4	4.34	neg	neg	present	acceptable
5	4.35	neg	neg	present	acceptable
6	4.50	neg	neg	present	acceptable
7	5.20	neg	neg	present	acceptable
8	3.97	neg	neg	present	acceptable
9	3.80	neg	neg	present	acceptable
10	4.36	neg	neg	present	acceptable
11	6.40	neg	neg	present	acceptable
Range	3.4-6.1	neg	neg	present	acceptable

GC-MS analysis (Table 6) revealed in three samples potentially harmful substances: A fragrance musk ambrette in sample 3 shows neurotoxicity in humans and in experimental animals. It induces murine neurological disease in humans (Spencer *et al.*, 1984), and is the cause of persistent light reaction in many patients (Goh and Kwok, 1986).

Musk ambrette has been found to produce hand limb weakness when administrated in the diet or applied to skin of rats (Spencer *et al.*, 1984). A fragrance versalide found in sample 10 is a compound either of natural or more often of synthetic origin. Versalide causes acute toxicity of thouguiacol in rodents (Butterworth and Mason, 1981). Versalide also shows medium cytotoxicity (lC50 = 0.16 mM) and is ranked 122 in Registry of Cytotoxicity out of

347 compounds tested. A fragrance menthofuran was found in sample 8. Its toxicity was reported in rats (Madyastha and Raj, 1991, Madyastha and Raj, 1992, Madyastha and Raj, 1994). Menthofuran, the metabolite of pulegone, causes toxicity to infants (Bakerink *et al.*, 1996).

Table 6
The results of examination for organic compounds.

Sample	Organic compounds
1	Syringaldehyde, menthofuran, trimethoxybenzyl methyl ether
2	Syringaldehyde, trimethoxybenzyl methyl ether
3	Musk ambrette, trimethoxybenzyl methyl ether, phenolic derivatives
4	Hyacinthin (phenylacetaldehyde)
5	Dihydromethyljasmonate
6	Syringaldehyde, trimethoxybenzyl methyl ether
7	Maltoxazine, , trimethoxybenzyl methyl ether
8	Hyacinthin, menthofuran, , trimethoxybenzyl methyl ether
9	Dihydromethyljasmonate, trimethoxybenzyl methyl ether, aspidospermine derivatives
10	Trimethoxybenzyl methyl ether, versalide
11	Pyran-4-one derivatives

Beside harmful organic compounds. some honey samples contained distinct amounts of compounds syringaldehyde, syringic acid aspidospermine, methyl jasmonate, phenylacetaldehyde, and trimethoxybenzoic acid. Aromatic aldehyde, syringaldehyde, in sample 1, 2, 6 shows antimicrobial activity against bacteria and fungus (Kobayash *et al.*, 1994,). Inhibition of flatoxin biosynthesis by phenolic compounds such as syringaldehyde was reported by Hua *et al.* (1999). Syringic acid, inhibited the peroxdatic activity of human ferricyanohemoglobin (Aymard *et al.*, 1975). Trimethoxybenzoic acid in samples 1-3 , 7-10 has antibacterial and antimycotic activity (Bisignano *et al.*, 2000). Also, Two bacteria strains were able to biodegrade seven of nine aromatic compounds, but 2,6-dihydroxybenzoic acid and 3,4,5-trimethoxybenzoic acid were the two undegraded compounds(Di Gioia *et al.*2001). Thus, because of trimethoxybenzoic acid and syringic acid (or their conversion products) have antimicrobial activity and may contribute to antimicrobial activity of honey.The indolalkaloides aspidospermine in sample 9, have been found to possess andrenergic blocking activities for a variety of urogenital tissues (Deutsch *et al.*, 1994). Vincadifformine, of the aspidospermine group, is acytotoxic and has antitumor effects (Sturdikova *et al.*, 1986). Jasmonate is a fatty acid derivative synthesized by plants. Dihydromethyljasmonate was found in sample 5. Methyl jasmonate was found to induce apoptosis in human cancer (Fingrut and Flescher, 2002) and may act as endogenous suppression for plant defense response inducing hypersensitive reaction (Andi *et al.*, 2001).

In summary, it may be stated, that honey samples 3, 8, and 10 are not recommended for human use due to the presence of toxic fragrance. Samples 1 and 7 were adulterated with sucrose and therefore are not fully authentic. On the other hand, samples 2, 4, 5, 6, 9 and 11 are suitable for human use.Our study demonstrates the necessity for the Saudi legislation to impose an analysis of imported honey, especially the constituents and the elements of the honeys.

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