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Physicochemical characteristics of some Egyptian honey from different botanical origins

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Abstract:

Eight of honey types were collected from different apiaries located in Egypt country during seasons of year 2018, depending on floral sources, banana (*Musa* sp.), bardkoush (*Origanum majorana*), camphor (*Cinnamomum camphora*), mesteka (*Pistacia lentiscus*), sidr (*Ziziphus spina-christi*), black seed (*Nigella sativa*), north Egyptian cotton Giza 94, 86 and upper Egyptian cotton Giza 90,95 (*Gossypium barbadense*). Pollen investigate of honey samples showed a wide variability, with samples from different honey sources being collected from geographical origins. The tested parameters viscosity, specific gravity, moisture content, electrical conductivity, total soluble solids, pH, lactone, free acidity, Total acidity, proline content, HMF and sugar (Fructose, glucose, sucrose, and maltose) are useful to determine the botanical origin of Egyptian honeys and their quality. The present study concluded that, the quality and physicochemical properties of honey were varied based on the geographical and botanical origins

Introduction

Determination of the standard criteria of food products is the most important process, since consumption, quality and validity of these products depend on it. Honey is one of the most important global natural products. Honey comes in the first order of these products, since it has many benefits in foods, and medicine (Hassan, 1985). Honey is defined as the natural sweet substance produced by honey bees from the nectar of plants, or from secretions of living parts of plants, or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honeycomb to ripen and matured (Rodriguez *et al*., 2004). Honey is always a mixture of different sources and no honey is completely the same as another (Oddo and Bogdanov, 2004). Honey contains approximately carbohydrates 80% (glucose 35 %, fructose 40 %, and sucrose 5 %) and water 20 %, serving as an excellent source of energy. In addition, it constitutes more than 200 components, including amino acids, vitamins, minerals, enzymes, organic acids, and phenolic compounds (Rodriguez *et al*., 2004 and Kahraman *et al*., 2010). Pollen investigation is the official method for the botanical origin determination of honey (Noaman *et al*., 2004). Properties and compositions of bee honey depend on its geographical floral origin, season, environmental factors and treatment of beekeepers (Kasˇkonien *et al*., 2010 and El-Metwally, 2015). The identification of honey plant sources is a subject of a great deal of interest since many years. There are various reasons why the floral origin of honey may be wanted to be known, such as, for quality control in marketing and where there is regulatory concern about the country of origin of honey (Molan, 1998).

This study aims to identify the authenticity and investigating the safety of representing various types of honey products sold in Egypt (24 samples). For this purpose, physicochemical properties, pH, HMF, and pollen test were performed. Sugar composition was also evaluated by means of high-performance liquid chromatography (HPLC) technique. All results were assessed based on Egypt standards, Codex Alimentarius Commission (CAC) (2001).

Materials and methods

1. Honey samples:

Twenty four samples of honey were harvested from apiaries located in different regions of Egypt during seasons of the year depending on floral sources , banana (*Musa* sp.), bardkoush (*Origanum majorana*) , camphor (*Cinnamomum camphora*), mesteka (*Pistacia lentiscus*), sidr (*Zizyphus vulgaris*), black seed (*Nigella sativa*) , North Egyptian cotton Giza 94,86 and upper Egyptian cotton Giza 90,95 (*Gossypium barbadense*) (Table,1). Honeys were collected from different location in Egypt regions were Ismailia, Kafr El-Sheikh, Beni Sweif, El-Minia and Assuit. Honey samples were collected in dark jars kept in freezing conditions until analyses.

Table (1): Types and floral sources of Egyptian honeys.

No. of samples	Local or English name of honey	Floral sources
Sample 1	Banana	*Musa* sp.
Sample 2	Bardkoush	*Origanum majorana*
Sample 3	Camphor	*Cinnamomum camphora*
Sample 4	mesteka,	*Pistacia lentiscus*
Sample 5	Sidr	*Ziziphus spina-christ*
Sample 6	black seed	*Nigella sativa*
Sample 7	north cotton	*Gossypium barbadense* (Giza94,86)
Sample 8	upper cotton	*Gossypium barbadense* (Giza90,95)

2. Physical properties:

2.1. Viscosity of honey was measured according to (Munro, 1943), the **specific gravity** was measured according to Wedmore (1955).

2.2. Determination of Color: The optical density of all the samples was determined and the color was measured by using the relation between optical density and USDA standards, as indicated by White (1978).

2.3. Determination of electrical conductivity (EC): According to the method of Vorwhol (1964).

3.Chemical properties:

3.1. Determination of moisture content: Determination of moisture content of honey was carried out by measurement its refractive index value (Abbe refractometer at 20ºC) **(A.O.A.C, 1995).**

3.2. Determination of total soluble solids (TSS) of honey by (**Association of Official Analytical Chemists** (**A. O. A. C.)**, **1980**). Equipment: Abbe refractometer was used and expressing the T.S.S. in honey in percentage.

3.3. Determination of pH, free acids, lactone content and total acidity

according the method of **White *et al.* (1962)**.

3.4. Determination of Proline content in honey samples. The proline content was measured according to (**Association of Official Analytical Chemists** (**A. O. A. C.)**, 1990).

3.5. Determination the quantity of sugars by High Performance Liquid Chromatography (HPLC). The concentration of fructose, glucose, sucrose and maltose in honey samples were determined by HPLC according to the method of Bogdanov and Baumann (1988).

3.6. Determination of Hydroxymethyl furfural (HMF). Hydroxymethyl furfural (HMF) was determined by using the standard method **Association of Official Analytical Chemists** (**A. O. A. C.) (1990)** Official Method 980.23.

3.7. Determination of pollen sediment content, according to the method of **Louveaux *et al*. (1978).**

Results and Discussion

1. Physical properties of honey:

Data in (Table, 2) showed some physical properties of honeys under investigation. The viscosity of honey types were ranged between 13.60±05 to 69.00±11 poise, there were significant differences among honey types, while no difference was recorded between the honeys of black seed and camphor also between banana and mesteka ,sidr and upper cotton .From the previous results it could be observed that the viscosity value of caplets was near to the maximum range in comparison with the normal values , while the other kinds recorded high values more than normal one , this may be due to the dried and hot atmosphere at the site where the caplets was planted that the high temperature degrees increase the values of this property .As pointed out by (White, 1975) the variations in viscosity of honey types are due primarily to temperature and water content where the values were highly different recording: 2.6, 10.7, 21.4 63.4 189.6 and 600 poise. Thawley (1969) and Crane (1990) related high viscosity of honeybee content of water, and (Pierro, 1994) reported that the viscosity is reduced when the temperature raises to 30°C. Moreover, Abd-EI-Bary and Meshrif (1993) found that the viscosity in clover and cotton honeys were 24.34 and 31.52 poise , respectively , where Meshrif *et al*. (1997) found that the viscosity of clover and cotton and sunflower honeys were 55.56, 63.48 and 116 poise .respectively . Al-Arify (1998) found that viscosity of some Saudi Arabian honeys ranged between 103.86 - 367.71 CP with mean value of 229.88 CP at 40°C.

1.1. Specific gravity in all honey types were nearly equal 1.40 it was ranged between 1.390±05 to1.42±0.36 with no significant differences. Regarding to specific gravity values at all tested honeys Table (2), it was noticed that, these values agreed with the normal degrees and fall within those found by White (1975); ranging between 1.421 to 1.423. Al-Arify (1998) found that specific gravity of Saudi honeys ranged from 1.42 to 1.44 with mean value of 1.432. Also, this result agrees with (El-Sharawi *et al*., 2009) that the specific gravity ranged between 1.39 to 1.42.

1.2. Electrical conductivity (EC):

As show in table 2, EC ranged between 110.0 ±10 to 520.0 ±10 ppm with significant differences among honey samples, while no difference was recorded between the honeys of black seed and bardakosh also between mesteka and sidr honeys ($P < 0.05$). EC is a good criterion of the botanical origin of honey and it is determined in routine honey control instead of the ash content (Adenekan *et al*., 2010). This measurement depends on the ash and acid content of honey, the higher ash and acid content, the higher the resulting conductivity. There is a linear relationship between the ash content and the EC. As for EC% it could be concluded that all tested honeys agreed with the ideal one. These results were less than Meshrif *et al.* (1997) who found that the electrical conductivity of Egyptian honeys was (0.45, 0.72, 0.87%) for clover, cotton and sunflower, respectively. The high EC values are attributed to high minerals content (Nour, 1988). Laurino and Gelli (2002) found that electrical conductivity of citrus honey was 0.185%. Tharwat and Nafea (2006) recorded that the EC ranged between 0.01 to 0.09 in some Saudi Arabia Honeys.

1.3. Color [Optical density (OD)]:

The color of honey usually ranges from light yellow to amber, dark amber and black in extreme cases and sometimes even green or red hues Bogdanov *et al*. (2008). Data presented in Ttable 2, showed that the color range of the eight Egyptian honeys was from 0.02 ±0.01 to 0.38±0.01 OD, the minimum value was detected in black seed honeys, while the maximum was detected in the banana honeys. There were significant differences among honey types, while no difference was recorded between the honeys of north cotton and upper cotton also between bardakosh and sidr honey.

Changes in color might be attributed to beekeeper's interventions and different ways of handling the combs such as the use of old wax combs for producing honey, minerals content contamination of heavy metals and exposure to either high temperature or light (El-Banby *et al*., 1989; Moniruzzaman *et al.,* 2013 and El-Metwally, 2015). Color classification of monofloral honeys is very important for commercial activities. The pruned value of Saudi and Kashmiri honey is like Gelam and Manuka honeys, which were amber, with pruned values of 122 and 110 respectively Moniruzzaman *et al.* (2013). According to the mentioned measures, it could be concluded that banana honey contains high ash than other honeys.

2.Chemical properties of honey:

2.1. Moisture content:

Data in Table (3), revealed that the moisture percentages of honey samples ranged between 17.25±0.66 to 21.0±1.11%, the lowest percentage was found in camphor honey and black seed honey, while the highest percentage was found in mesteka honey. There were significant differences among honey types, while no difference was recorded between the honeys of sidr, north cotton and upper cotton ($P < 0.05$). The higher the moisture content is the higher probability of honey fermentation during storage (Singh and Bath, 1997). Lower moisture limits (<20%), elongates honey shelf life which would be met by a large majority of the commercial honeys (Terrab *et al*., 2003). These results were accepted by the international regulations for honey quality (Codex Alimentarius Commission (CAC) , 2001) and Council Directive of the European Union, 2001). However, moisture content depends on the temperature and relative humidity in the geographical origin during honey producing in honey colonies (Crane, 1979). Moisture content is an important quality parameter, important above all for honey shelf-life (Bogdanov *et al*., 2008).

These results are in symmetry with the values obtained by Sancho *et al*. (1991) mentioned that the moisture content ranges from 12.4 to 20.3 %, Foldhazi (1994) reported a range of 16.46 to 17.70 %, while Ihtishamulhaq *et al.* (1998) reported higher ranges of 17.6 to 21.83 %. Finally, Al-Arify (1998) found that moisture of Saudi honey ranged from 14 to 16.9 % with mean value 15.26%. El-Sharawi *et al.* (2009) found that the moisture content ranged from 17.5 to 23.0% in honeys collected from different location in Aswan.

2.2. Total soluble solids (TSS):

Percentage of honey samples ranged between 79.0±0.7 to 87.75±0.92%. It could be noticed that all honey content of TSS located at the normal rate of honeys. In table 2, showed that the lowest percentage of honeys (79.0 %) was found in mesteka honey, while the highest percentage (87.75 %) was found in black seed honey. There were significant differences among honey types. While no difference was recorded between the honeys of sidr, banana and upper cotton (P<0.05) (Table, 3). The TSS which should be 77% or more, is responsible for protecting honey from fermentation. In this respect, these results are in harmony with those obtained by Minh *et al*. (1971) who reported that 79.34 % TSS was recorded in honeys from Philippines. Hussein (1989) mentioned 76.83 % TSS in honey from Oman, and finally, Al-Arify (1998) found that the TSS of Saudi honey ranged from 81.73 to 84.33 % with mean value 83.26%.

As for the values of pH, it could be concluded that all collected honeys recorded pH values ranged between 3.7±0.17 to 4.7±0.26 found within the normal values of honeys (3.42 to 6.1). All tested samples were acidic table 3, and within the standard limit (pH 3.40 to 6.10) (Codex Alimentarius Commission (CAC), 2001) that insures honey samples' freshness. There were no significant differences among all honey types, except for sidr honey recorded highly significant value 4.7 (P <0.05).The pH values of four tested types of honey samples were close to those previously reported in Indian, Algerian, Brazilian, Spanish and Turkish honeys (between pH 3.49 and 4.70) (Azeredo *et al*., 2003; Ouchemoukh *et al*., 2007; Kayacier and Karaman, 2008 and Saxena *et al*., 2010). The high acidity of honey correlates with the fermentation of sugars present in the honey into organic acid, which is responsible for two important characteristics of honey: flavor and stability against microbial spoilage (Bogdanov *et al*., 2008). Furthermore, it might also indicate that the honey samples have high content of minerals (Mohammed and Babiker, 2009 and El-Metwally, 2015).

Acidity in honey is calculated as free acidity, lactonic and total acidity. Specifics a free acidity of not more than 50 meq/1000 g (meq/kg) (European Commission, 2002). Some factors affecting bee honey acidity e.g. harvest seasons and floral types (El-Sherbiny and Rizk, 1979 and Pe´rez-Arqullue *et al*., 1994). The ranged values for free acidity in honey samples between 11.0±1.32 to 68.3±0.85 (meq/kg). There were significant differences among all honey types, except for banana honey recorded highly acidity significant value 68.3 (P =0.000) (Table,3). Lactonic acid ranged from 7.5±0.7 to 17.5±0.70 meq/kg and found highly significant between all samples (P=0.000) (Table, 3). Total acidity detected highly significant between all samples (P=0.000 (Table, 3), it's ranged from 18.51. ±1.05 to 86.0±0.7 meq/kg; The present investigations are quite in agreement with Ouchemoukh *et al*. (2007).

2.3. Sugar (Fructose, glucose, sucrose, maltose) content of collected honey samples indicated that most of tested samples contain ideal values representing normal values of honeys. In addition, it could be observed that all tested samples of fructose sugar were ranged between (38.2±0.66 to 41.2±0.30%) while the normal content is (42.5 to 50.8%). There were significant differences among honey types. And no difference was recorded between the honeys of camphor, banana and upper cotton (P <0.05) (Table,3). Glucose values of all tested honeys were ranged between (28.0 ±1.23 to 32.0±1.61%), it means that the honey content of glucose is partially like normal ones. There were significant differences among honey types, while no difference was recorded between the honeys of black seed, mesteka and north cotton (*P* <0.05) (Table, 3).

Regarding to sucrose values of all tested honeys, it was ranged between (1.1±0.09 to 5.1±0.30%), it means that the honey content of sucrose is partially like normal ones. There were significant differences among honey types, while no difference was recorded between the honeys of black seed and north cotton and between camphor, bardakosh and sidr honey (*P* <0.05) (table3). The international normal established by Codex Alimentarius Commission (CAC) (2001) that a good quality honey should not contain more than 5 % sucrose. The values obtained for sucrose contents of the honey samples were all within the limits of international standards. According to White and Doner (1980), the sucrose level in honey never arrives at zero. The sucrose contents obtained in this realization are within the range of values stated for Argentine and Turkish (Cantarelli *et al*., 2008), Venezuelan (Vit *et al*., 2009), American (White and Doner, 1980), Algerian (Makhloufi *et al*., 2007), Pakistani (Zafar *et al*., 2008) and Spanish (Cavia *et al*., 2006) honeys.

As for, maltose values of all tested honeys, it was ranged between (4.5±0.20 to 10.0±0.62%), it means that the honey contents of maltose sugar are within the normal values. The statistical analyses show significant differences among honey types, while no difference was recorded between the honeys of black seed, mesteka, north cotton and upper cotton and between camphor, bardakosh honey (*P* <0.05) (Table,3). Comparable results are reported by the previous several studies on different honey types (Buba *et al*., 2013 and El-Metwally, 2015). Fructose/ glucose ratio (F/G) indicates the ability of honey to crystallize. F/G ratio of honey samples were ranged from 1.45 to 1.9 and the glucose/water (G/W) ratio of honey samples were ranged from 1.25 to 1.4(Table, 3). White and Doner (1980) noticed that even though honey has less glucose than fructose, the honey was granulated because glucose less soluble in water than fructose. When the F/G ratio is high, honey remains liquid. Honey crystallization is slower when the F/G ratio is more than 1.3 and it is rapid when the ratio is below 1.0. However, the G/W ratio is considered more suitable than the F/G ratio for the forecast of honey crystallization. It has been stated that when the G/W ratio is <1.3 honey crystallization is very slow or even zero and it is complete and rapid when the ratio is >2.0 (Amir *et al*., 2010). Glucose, which is a major sugar in honey, can spontaneously crystallize from honey solutions in the form of its monohydrate (White and Doner, 1980). This sometimes occurs when the moisture level in honey can drop below a certain level; i.e., when the moisture content is very low.

2.4. Amino acid proline content of honey samples were ranged from 316.67 ±8.01 to 566.7±2.05ppm, the statistical analyses shows significant differences among honey types, while no difference was recorded between the honeys of bardakosh and sidr (*P*<0.05) (Table,3). From the foregoing findings it could be concluded that proline is the predominant essential amino acid in floral and non-floral honeys, the literature contains variable results regarding the amino acids distribution in multifloral honeys from different geographical areas (White, 1975).

Data in Table (3), indicated to HMF concentrations of the honey samples ranging from 2.0±0.17 to

23.04±0.30 mg/kg Notably all HMF concentrations were within the recommended range set by the Codex Alimentarius Commission (CAC) (2000) at 80 mg/kg. The values are also within the allowed maximum limit of 40 mg/kg, as recommended by the Turkish Alimentarus Codex Commission (2003) for honey samples from tropical countries. The HMF content, which is used as an index of heat treatment of honey, indicated that this honey with highest HMF. The accumulation of HMF was due to processing of honey at high temperature above 75°C or storage above 27°C for months Turkish Alimentarus Codex Commission (2003). Analysis of variance of HMF reveals that there is a significant difference among HMF of different honey (Table,3), except for black see , camphor and bardakosh honey shows no significant differences between them (P <0.05).Overall, the low HMF concentrations of the tested Egyptian honey confirm that these samples are of good quality.

Abselami *et al*. (2018) found that except for lavender honey that contained 56.14 mg/kg of HMF, the HMF concentrations of the remaining honey samples ranging from 3.98 to 38.55 mg/kg. Laurino and Gelli (2000) reported that the values of HMF ranged between 2.0 to 26.0mg/kg. Nour *et al.* (1991) found that HMFvalues ranged between 2.0 to 19.13mg/kg. In freshness honeys. Tharwat and Nafea (2006) found that HMF in Saudi honeys ranged between 0.48 to 21.12 mg/kg.

It is concluded that, the quality and physicochemical properties of honey were varied based on the geographical and botanical origins.

Table (2): Physical properties of different of some Egyptian honey types.

	Honey types							
Properties	**Black seed**	**Camphor**	**Banana**	**Bardkoush**	**Mesteka**	**Sidr**	**Cotton Nourth**	**Cotton Upper**
Viscosity (Poise)	69.0 ±0.11 a	69.0 ±0.05 a	20.0 ±0.10 e	48.1 ±0.05b	13.6 ±0.05 e	34.9 ±0.10 d	36.9 ±0.07 c	34.9 ±0.9 d
Specific gravity	1.42 ±0.36 a	1.42 ±0.53 a	1.4 ±0.53 a	1.4 ±0.30 a	1.39 ±0.50 a	1.41 ±0.56 a	1.41 ±0.50 a	1.41 ±0.89 a
Color	0.02 ±0.01 f	0.19 ±0.01 c	0.38 ±0.01 a	0.16 ±0.03 d	0.23 ±0.01 b	0.16 ±0.017 d	0.12 ±0.01 e	0.13 ±0.03 e
EC %	170.0 ± 5.00 e	200.0 ±21.7 d	520.0 ±10.0 a	170.0 ±10.0 e	380.0 ±10.0 b	470.0 ±5.00 b	110.0 ±10.00 f	260.0 ±0.50 c

Different letters indicate in the row significant difference (P<0.05).

Table (3): Chemical composition of some Egyptian honey types.

Parameters	Black seed (multifloura)	Camphor	Banana	Bardkoush	Mesteka (multifloura)	Sidr	Cotton North	Cotton Upper
Moisture (%)	17.25 ±0.66 cd	17.0 ±0.26 d	20.0 ±1.11 ab	18.5 ±0.70 bc	21.0 ±1.11 a	19.5 ±0.70 b	19.0 ±0.70 b	19.50 ±0.75 b
Tss (%)	87.75 ± 0.92a	83.0 ±0.50 b	80.0 ±1.00c d	81.5 ±0.50 c	79.0 ±0.7 d	80.5 ±1.00 cd	81.0 ±0.89 c	80.5 ± 0.04 cd
pH	4.1 ±0.20 b	4.1 ±0.30 b	3.7 ±0.17 b	3.8 ±0.20 b	4.1 ±0.36 b	4.7 ±0.26 a	3.9 ±0.30 b	4.0 ± 0.3 b
Free acidity	19.0 ±2.00d	11.0 ±1.32 f	68.3 ±0.85 a	21.0 ±2.65 d	33.5 ±101 b	16.0 ±0.70 e	13.5 ±1.32 ef	25.0 ±0.01 c
Lacton (meq/kg)	12.5 ±0.70 c	10.0 ±0.70 d	17.5 ±0.70 a	12.5 ±0.62 c	15.0±0.70 b	7.5 ±0.70 e	10.0 ±0.36 d	12.5 ±8.66 c
Total acidity	31.5 ±0.92 d	21.5 ±1. 05 f	86.0 ±0.70 a	33.5 ±0.92 c	18.5 ±1.05 g	23.5 ±1.05 e	23.5 ±0.53 e	37.5 ±1.0 b
Fractuse	41.2 ±0.30 a	38.5 ±0.40 e	38.2 ±0.66 e	39.9 ±0.44b c	40.5 ±1.05 ab	39.2 ±0.30de	39.5 ±0.40 cd	38.2 ±1.0 e
Glucose	31.00 ±1.67 ab	32.00 ±1.61 a	29.4 ±1.15 bc	28.00 ±1.23 c	31.1 ±0.87 ab	28.4 ±1.08 c	31.5 ±1.15 ab	30.1 ±0.2 abc
Sucrose	4.00 ±0.50 b	5.00 ±0.50 a	1.1 ±0.09 d	5.1 ±0.30 a	1.3 ±0.17 d	5.00 ±0.46 a	3.5 ±0.40 b	2.8± 2.65 c
Maltose	5.00 ±0.62 d	7.4 ±0.46 b	10.00 ±0.62 a	7.2 ±0.36b	4.5 ±0.20 d	6.1 ±0.36 c	4.5 ±0.5 d	0.46 ±0.3 d
Glucose/Water	1.8 ±0.12 ab	1.9 ±0.07 a	1.5 ±0.12 cd	1.5 ±0.07 cd	1.5 ±0.12 cd	1.45 ±0.07 a	1.7 ±0.01 bc	1.5 ±0.62 cd
Fructose/glucose	1.3 ±0.08 abc	1.2 ±0.05 d	1.3 ±0.03 bc	1.4 ±0.05 a	1.3 ±0.07 bc	1.4 ±0.01 ab	1.25 ±0.03 cd	1.3 ±4.41 cd
Proline (ppm)	366.67 +8.01 e	316.67 +8.01 g	450.0 +4.36 c	566.67 +8.01 a	550.0 +4.86 b	566.7 +2.05 a	350.0 +4.36 f	383.33 +0.46 d
HMF (ppm)	7.68 ±0.40 d	7.6 ±0.46 d	13.4 ±0.46c	7.7 ±0.46 d	17.3 ±0.40 a	2.0 ±0.17 f	23.04 ±0.30 a	5.7 ± 0.36 e

Different letters indicate in the row significant difference ($P<0.05$).

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