

Physical and chemical studies on the Egyptian honey compared with standard honey specification

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ABSTRACT

Egyptian honey standard of 1990 needs modifications to meet the demands of Egyptian honey producers and to agree with the growing requirements of the international market. A total of 35 honey samples of citrus, clover and cotton honeys were collected from 26 locations in Alexandria and Behera provinces. Samples were analyzed according to the official methods of analysis of the AOAC (1984) for the determination of sucrose, reducing sugars, moisture content, water insoluble solids, ash, free acidity, lactone, total acidity, diastase activity and hydroxymethylfurfural (HMF) concentration. The honey samples were compared with the standards of Codex (1998), Gulf (1992) and Egypt (1990). Pollen content was studied in all samples as well. The Egyptian honey standard was proven to be stricter than other standards and is recommended to be modified as the apparent sucrose content should be raised to 10 % except for the cotton honey, the reducing sugar contents should be 65 %, the ash content should be 0.6 g per 100 g honey, the HMF limit should be 80 mg / kg and the diastase activity should be included as 3 on Gothe scale.

Keywords: Honey, HMF, diastase number, reducing sugars, pollen, honey standards

INTRODUCTION

As one of the major products of honeybees, honey is processed by workers after its collection as flower nectar. It contains a variety of carbohydrates, amino acids, minerals, proteins (enzymes) and water (Qui *et al.*, 1999). Honey is therefore collected from different flower sources leading to variations in its physical-chemical characteristics as a final product (White, 1980).

Honey standardization has therefore, become an important issue to consider. Every country or a region of countries has suggested special honey standards, depending on different factors existing in such countries. These factors are attributed to environmental conditions, floral type, and individual beekeeping practices. In the past, USA standards judged honey brands by two methods, the first method was the grading, which was based on four factors: Soluble solids, flavor, absence of defects and clarity. While the second method was the color measured by the aid of many instruments as the refractometer, the Pfund grader and the US Department of Agriculture color comparator (El-Ansary, 1998).

Recently, standardization of the honey depends on major tests that have to be determined. They are, reducing sugar content, sucrose content, fructose: glucose ratio, moisture content, water insoluble solids content, ash, acidity, diastase activity and hydroxymethylfurfural (HMF) content. These tests require tremendous efforts to conduct on all honey brands within or between countries or regions.

The aim of this study is to evaluate physical-chemical characteristics of Egyptian honey products with a special reference to Egyptian honey standards.

MATERIALS AND METHODS

I- Materials:

The present work was carried out on 35 honey samples collected from Alexandria and Behera provinces. Samples were provided from either beekeepers or markets representing three types of honey namely citrus, clover, and cotton honey.

II- Methods:

Honey test methodologies were carried out according to those described by Codex Alimentarius Commission CL 1998/12-S, Recommended European Regional Standard for honey, Gulf Standard No. 122/1990 and Egyptian Standard No. 355/1990.

A. Honey Samples Preparation:

Liquid strained honey was suggested to be the form of all honey samples. A glass jar was used for packaging a 1000 g of honey sample till analysis.

Before weighing portions for determination, granules-free strained honey samples were used. Liquefying these samples was achieved by placing closed container in water bath for 30 minutes at 60 – 65 °C while shaking. Samples were mixed and cooled rapidly as soon as they were completely liquefied. Honey samples prepared for hydroxymethylfurfural or diastase determination were not heated.

For the removal of foreign materials like wax, sticks, bees and other particles, samples were heated to 40 °C in water bath and strained through cheesecloth.

B. Analytical Methods:

Honey samples were analyzed according to the standard methods of the Association of Official Analytical Chemists (AOAC, 1984).

1- Water content and refractive index:

The refractive index of honey was measured by refractometer and corrected to standard temperature at 20 °C (AOAC, 1984) then the corresponding moisture content was deduced from AOAC tables by using the values of refractive index at the designated temperature.

2- Water insoluble solids:

A honey sample of 20 g was weighed and dissolved in suitable quantity of distilled water at 80 °C and mixed well. Samples were filtered through dried, sintered glass crucible with pore size between 15-40 micron and washed well with hot water at 80 °C until it was free from chloride ion, that was indicated by the addition of 5 drops of silver nitrate solution (AgNO_3 0.1 %) for each 0.5 milliliter of filtrate until no turbidity occurred. The filtrate was dried at 35 °C for an hour, then cooled and weighed to the nearest 0.1 mg.

3- Ash content:

Five grams of honey samples was placed under an infrared lamp (375 watt) with variable voltage input, which slowly increased until the sample became black and dry. The samples were then placed in an electric muffle furnace at 600 °C. Samples were cooled and weighed. The results were expressed as W / W ratio (percent).

4- Determination of sugars:

1. Analysis of individual sugars by HPLC:

Two HPLC instruments were used in this study, one in Al-Asher men Ramadan city (M510, Waters, USA) and the other in the National Center of Agricultural Research (Shimadzu, Japan). Both were fitted with refractive index detectors (Atago, Rx 1000 & RID-10A, Japan). Instruments were also equipped with an Aminex carbohydrate column. The column temperature was set on 85 °C. Diluted honey samples of 1 ml at a concentration of 10 brix were injected. The mobile phase was methanol: deionized water (70:30) at 40 °C and a pressure of 500 psi with a flow rate of ml/min.

Glucose, fructose and sucrose percentages were automatically calculated from their peak dimensions and retention time. The actual sugars percentages were calculated using the following equations:

$$\text{Total Sugars\%} = 100 - (\text{Water content\%} + \text{Ash\%} + \text{Water insoluble solids \%})$$

$$\text{Actual sugar content \%} = (\text{Total sugars \%}) (\text{Area \%}) / 100$$

II. Determination of total sugars by Nelson arsenomolybdate colorimetric method:

Sugars were extracted from 5 g of honey samples using distilled water, according to Loomis and Shull (1937). Reducing sugars as well as the total sugars were determined before and after hydrolysis with hydrochloric acid as described by Malik and Singh (1980). The non-reducing sugars content was expressed as g / 100 g fresh weight of honey.

5- Determination of hydroxymethylfurfural (HMF):

Honey samples of 5 g were added to 25 ml H₂O in a 50 ml volumetric flask, then 0.5 ml of Carrez solution-I (15 g K₄Fe (CN)₆·3H₂O dissolved into 100 ml water) was added and mixed followed by 0.5 ml of Carrez solution-II (30 g Zn (OAc)₂·3H₂O dissolved into 100 ml water) and diluted to volume with water. A drop of alcohol may be added to suppress foaming and the mixture was filtered through paper. Five mls of the filtrate were pipetted into test tubes including control. Five mls of Sodium bisulfite solution (0.20 %) were mixed well to each sample, then absorbance was determined using UV-spectrophotometer (Ultrospec 3000, Pharmacia Biotech) at 284 and 226 nm using a 1-ml cell (AOAC, 1984). The amount of HMF was calculated using the following equation.

$$\text{HMF} / 100 \text{ g honey} = (A_{284} - A_{336}) \times 14.97 \times 5$$

6- Diastase activity:

One ml of 2 % starch solution was pipette into several 50 ml graduated cylinder containing 10 ml of diluted iodine solution (0.007 N) and mixed. The quantity of water necessary for dilution was determined to produce an absorbency value of 0.76 ± 0.02 . Five grams of honey samples dissolved in 10 ml water plus 2.5 ml acetate buffer solution at pH 5.3 were added to 1.5 ml NaCl solution (0.5 N) in a 25 ml volumetric flask and brought to volume by water. Five ml of starch solution and 10 ml of each sample were placed in a flask and kept in a water bath for 15 minute at 40 ± 0.2 °C. One ml was added to 10 ml of Iodine solution after 5 minute. Absorbency values were determined using Spectronic-20 (Bausch & Lomb) at 660 nm in 1 cm cells. Aliquots of 1 ml were taken continuously at intervals until the absorbency values reach less than 0.235. Diastase number (DN) was obtained by dividing 300 by the time taken to reach the absorbency value of 0.235 (AOAC, 1984).

7- Determination of free lactone and total acidity:

Ten grams of honey sample were added to 75 ml carbon dioxide free water in 250 ml beaker. The pH values were recorded using a pH meter (Led-model No. 5986, Coleparmer Instrument Co.). The free acidity was determined by titrating the tested sample with 0.05 N NaOH until pH 8.50. After the addition of 10 ml sodium hydroxide back titration with HCl 0.05 N to pH 8.3 was performed to determine lactones (AOAC, 1984). Total acidity was calculated as melliequivalent (meq) / kg of honey.

Free acidity = (ml 0.05N NaOH – ml Blank) X 50 / g sample.

Lactone = (10.0 – ml 0.05N HCl) X 50 / g sample.

Total acidity = Free acidity + Lactone.

8- Pollen grains analysis:

Pollen slides were prepared using the acetolysis method described by Eredtman (1954) and modified by El-Ansary (1981).

For inspection of the pollen grains in honey samples, 5 ml of from the upper part of the honey jar were diluted in hot water, centrifuged and suspended in sulfuric acid which was added slowly to nine-volumes of acetic anhydride. The whole volume was heated while stirring and then centrifuged (2200 / min). Pellets were washed with water several times.

Drops of glycerin were added and pellets were re-centrifuged and filtered on paper over night at 20 °C. Some pollen grains became dark as a result of acetolysis, these had to be bleached before microscopic examination.

Statistical analysis: A randomized block design was used and analysis of variance was carried out using Superanova® (Abacus Concepts, Inc., Berkeley, California, U.S.A). Significant differences between means were determined by Fisher's LSD Test at $P = 0.05$.

RESULTS AND DISCUSSION

Data of all honey samples were evaluated and compared with the international honey standards of the Codex (1998), Saudi (1990), Gulf (1992) and the Egyptian standard (1990).

1- Moisture content:

According to its moisture content, honey samples could be divided into two groups. The first group includes all samples accepted by the four honey standards (below 20 %), which have no risk for yeast fermentation within a year. Ten samples of citrus honey, twelve of clover honey and eleven of cotton honey were recorded in this regard. The lowest water content values recorded was 15.40 %, while the highest was 19.40 %.

The second group includes three samples with a moisture content of 20 to 23 %, which are not accepted by the Egyptian standard (1990). These samples were Al-Tarh citrus honey (22.00 %), Khorshid citrus honey (21.40 %) and Edeco cotton honey (20.60 %). In the mean time, the first two samples were not accepted by the international standards.

Water content was reported to be about 23 % in honey samples collected from Kerala, India, (Natarajan and Yesuvadin, 1978), 18 % in samples of honey from Iraq (Al Naji and Hujazy, 1982), 14 – 17 % in Connecticut, USA (Hankin 1987), 17 – 20 % in Spain (Sanz *et al.*, 1995) and 16.1 % in Austria (Kohlich *et al.*, 1995). In this respect, Al Naji and Hujazy (1982), Sanz *et al.*, (1995), Russmann (1998) and Bogdanov (1999) indicated that honey with relatively high moisture content (more than 18 %) may undergo yeast fermentation leading to a rapid increase in the number of yeast and bacteria in honey.

2- Water insoluble solids:

The water insoluble solids are mainly wax sediment, foreign matters and fragments of insects. Most of honey samples were accepted by the four standards. Samples did not exceed the upper limit, which was 0.5 %. Only 8 samples were not accepted having between 0.57 – 0.9 %. They were 3 of citrus honey, one of clover honey and 4 of cotton honey. Water insoluble solids were estimated to be less than 0.1 % in Australian Regulations (1993).

3- Ash content

Except for the cotton honey sample of Ezbit El-Shikh (0.9 %), all honey samples were accepted by the four honey standards, which indicates that ash content did not exceed 0.6 %.

Ash content was estimated in Turkey to be between 0.14 – 0.56 % (Temiz, 1983), while in the USA was between 0.04 – 0.63 % (Hankin, 1987). Meanwhile, Rybak and Achremowicz (1986) found that very low ash content may be an indication of honey adulteration.

4- Reducing sugars content:

Reducing sugars content Data (Tables 1, 2 and 3) showed that honey samples could be divided into three groups. The first group includes all samples accepted by the four honey standards, which is above 70 %. They included 8 citrus honey samples, 6 clover honey samples and 11 cotton honey samples. The highest value was 78.58 % (Al-Sabahia cotton) while the lowest was 71.77 % (Damanhour citrus).

The second group included samples that do not agree with the Egyptian standard, but accepted by other international standards. In other words, the second group of honey samples has values of reducing sugars between 70 % and 65 %. These samples are citrus honey of Kom-Hamada, Al-Tarh and Magdy, clover honey of Kafr El-Dawar and Al-Tabia. The previous honey samples have reducing sugars values of 68.39, 65.37, 65.57, 69.99 and 67.60 %, respectively.

The third group included honey samples that have less than 65 % and subsequently did not match all standards. These samples are citrus honey of Abis, clover honey of Ezbit El-Shikh, Al-Abaadia, Miser Polan and cotton

Table (1): Composition of citrus honey samples from different locations in comparison with the four used international standards.

Location of samples	Moisture %	Water-insoluble solids %	Ash %	Reducin g sugars %	Apparent Sucrose %	HMF* mg/kg	DN*	Total acidity meq/kg	Lactone meq./kg	Free acidity meq/kg	pH
Kom-Hamada	19.40 ^f	0.109 ^e	0.168 ⁱ	68.39 ^c	9.24 ^b	33.23 ^k	6.3 ^c	24.54 ^{ph}	2.51	22.03	3.53 ^c
Damanhour	19.20 ^b	0.204 ^f	0.157 ^m	71.77 ^b	8.67 ^b	19.46 ⁱ	9.5 ^a	27.28 ^f	2.26	25.02	3.47 ^{cd}
Baslacon	15.60 ^d	0.132 ^j	0.179 ^j	77.75 ^a	4.09 ^d	75.75 ^c	4.8 ^b	32.30 ^{cd}	1.43	30.87	3.42 ^{cd}
Edfina	19.60 ^b	0.141 ⁱ	0.203 ^h	73.52 ^b	6.54 ^c	76.96 ^d	8.8 ^b	29.29 ^e	1.21	28.08	3.33 ^{cd}
Khat Westany	16.60 ^{cd}	0.900 ^a	0.279 ^e	76.92 ^a	5.30 ^{cd}	74.55 ^f	5.7 ^e	30.96 ^d	1.41	29.55	3.34 ^{cd}
Al-Maamora	17.40 ^c	0.892 ^b	0.217 ^g	75.49 ^{ab}	6.00 ^{cd}	48.80 ^j	7.5 ^d	32.80 ^c	1.32	31.48	3.41 ^{cd}
Al-Tarh	22.00 ^a	0.191 ^h	0.178 ^k	65.37 ^d	9.16 ^b	85.18 ^b	8.8 ^b	28.37 ^{ef}	1.32	27.05	3.37 ^{cd}
Abis	19.80 ^b	0.124 ^k	0.220 ^f	60.23 ^e	17.28 ^a	62.57 ^h	7.8 ^c	29.29 ^e	1.32	27.97	3.3 ^{cd}
Khorshid	21.40 ^{ab}	0.690 ^c	0.137 ⁿ	72.12 ^b	5.65 ^d	64.20 ^g	6.0 ^f	30.32 ^{de}	2.00	28.32	3.43 ^{cd}
Kafir El-Dawar	16.51 ^{cd}	0.412 ^e	0.190 ⁱ	73.24 ^b	4.80 ^d	88.62 ^a	8.7 ^b	31.30 ^d	1.20	30.10	3.33 ^{cd}
Magdy	17.41 ^c	0.102 ^l	0.550 ^c	65.57 ^c	16.38 ^a	75.60 ^e	3.4 ^j	24.78 ^f	1.83	22.94	3.33 ^{cd}
El-Nahal	15.40 ^d	0.302 ^e	0.560 ^b	75.10 ^{ab}	8.65 ^b	35.93 ^j	3.8 ⁱ	23.17 ^h	1.50	21.67	3.20 ^d
Standards											
Egyptian standard	20 ^h	0.5 ^d	0.4 ^d	70.00 ^e	5.00 ^{cd}	40 ^j	-	-	-	-	4 ^b
Codex standard	21 ^{ab}	0.5 ^d	0.6 ^a	65.00 ^d	10.00 ^b	40 ^j	3 ^k	50 ^a	-	-	-
Gulf standard	21 ^{ab}	0.5 ^d	0.6 ^a	65.00 ^d	10.00 ^b	80 ^c	3 ^k	40 ^b	-	-	5.6 ^a
Saudi standard	21 ^{ab}	0.5 ^d	0.6 ^a	65.00 ^d	10.00 ^b	80 ^c	3 ^k	40 ^b	-	-	-

• HMF = Hydroxymethylfurfural (mg/kg), DN = Diastase number according to (Gothe-Scale number).

• Means at each column followed by the same letter (s) are not significantly different at p=0.05.

Table (2): Composition of clover honey samples from different locations in comparison with the four used international standards.

Location of Samples	Moisture %	Water-insoluble solids %	Ash %	Reducin g sugars %	Apparent Sucrose %	HMF* mg/kg	DN*	Total acidity meq/kg	Lactone meq/kg	Free acidity meq/kg	pH
Khorshid	15.40 ^f	0.201 ^e	0.198 ⁱ	79.92 ^a	4.28 ^{de}	42.51 ^f	8.1 ^b	40.83 ^d	10.83	30.00	3.42 ^f
Ezbit El-Shikh	17.20 ^d	0.090 ^k	0.158 ^k	61.36 ^e	4.82 ^{de}	16.17 ^k	8.4 ^a	32.69 ⁱ	8.00	24.69	3.75 ^{ed}
Baslacon	15.40 ^f	0.098 ^j	0.315 ^e	78.30 ^a	4.50 ^{de}	39.37 ^g	5.0 ^e	36.83 ^g	9.17	27.67	3.71 ^d
Abis	19.40 ^c	0.101 ^h	0.433 ^d	76.25 ^{ab}	3.82 ^{de}	38.32 ^h	7.0 ^c	44.00 ^c	10.48	33.52	3.77 ^e
El-Monofia	15.40 ^f	0.322 ^d	0.355 ^f	77.70 ^{ab}	5.96 ^d	40.26 ^g	6.1 ^f	47.27 ^b	12.75	34.52	3.70 ^d
El-Nozha	17.40 ^d	0.924 ^a	0.157 ^k	76.67 ^{ab}	0.67 ^f	51.80 ^d	3.8 ^b	38.25 ^f	9.83	28.42	3.65 ^e
KaferEl-Dawar	19.40 ^c	0.099 ⁱ	0.546 ^c	69.99 ^c	9.97 ^c	40.27 ^g	8.1 ^h	37.35 ^g	9.17	28.18	3.64 ^e
Al Abadia	19.40 ^c	0.136 ^g	0.138 ⁱ	63.51 ^{de}	15.14 ^a	81.29 ^a	6.3 ^e	35.92 ^h	8.83	27.08	3.75 ^{ed}
Al-Tabia	16.60 ^e	0.438 ^c	0.177 ^j	67.60 ^e	12.57 ^h	75.90 ^c	6.8 ^d	34.58 ^h	8.33	26.25	3.72 ^d
Damanhor	19.40 ^c	0.147 ^f	0.219 ^h	74.62 ^b	3.32 ^e	46.56 ^e	7.0 ^c	39.58 ^e	10.83	28.75	3.43 ^f
Miser-Polan	16.60 ^e	0.099 ⁱ	0.569 ^b	60.09 ^e	16.40 ^a	24.70 ⁱ	1.4 ^j	34.83 ^h	7.00	27.83	3.34 ^e
Standards											
Egyptian standard	20 ^b	0.5 ^b	0.4 ^e	70.00 ^c	5.00 ^d	40 ^g	-	-	-	-	4 ^b
Codex standard	21 ^a	0.5 ^b	0.4 ^a	65.00 ^d	10.00 ^c	80 ^h	3 ⁱ	50 ^a	-	-	-
Gulf standard	21 ^a	0.5 ^b	0.6 ^a	65.00 ^d	10.00 ^c	40 ^g	3 ⁱ	40 ^c	-	-	5.6 ^a
Saudi standard	21 ^a	0.5 ^b	0.6 ^a	65.00 ^d	10.00 ^c	40 ^g	3 ⁱ	40 ^c	-	-	-

- HMF = Hydroxymethylfurfural (mg/kg). DN = Diastase number according to Gothe-Scale number).
- Means at each column followed by the same letter (s) are not significantly different at p=0.05.

Table (3): Composition of cotton honey samples from different locations in comparison with the four used international standards.

Location of samples	Moisture %	Water-insoluble solids %	Ash %	Reducing Sugars %	Apparent Sucrose %	HMF* mg/kg	DN*	Total acidity meq/kg	Lactone meq/kg	Free acidity meq/kg	pH
Abo-Homous	19.00 ^f	0.233 ⁱ	0.443 ^{ef}	75.92 ^b	3.20 ^d	62.87 ^c	7.1 ^b	36.33 ^d	20.17	23.72	3.64 ^{cd}
Baslacon	19.40 ^c	0.195 ⁱ	0.455 ^c	74.12 ^b	5.84 ^c	33.53 ^b	9.2 ^a	46.65 ^{ab}	26.30	30.02	3.53 ^d
Sidy Ghazy	19.60 ^d	0.310 ^h	0.218 ^j	75.47 ^b	4.40 ^{cd}	48.50 ^f	7.3 ^b	19.85 ^e	11.95	9.20	3.95 ^b
Maatuok	18.80 ^e	0.621 ^c	0.546 ^c	75.70 ^b	4.34 ^{cd}	30.24 ⁱ	7.4 ^b	36.42 ^d	20.67	23.17	3.87 ^{bc}
Edco	20.60 ^b	0.199 ^j	0.489 ^d	73.74 ^b	4.05 ^{cd}	45.81 ⁱ	8.0 ^{ab}	41.67 ^c	22.20	26.17	3.95 ^b
Al-Sabbia	18.20 ^h	0.810 ^b	0.351 ^h	78.58 ^a	0.26 ^e	48.80 ^f	7.5 ^b	45.35 ^b	24.00	29.69	3.53 ^d
Azbit El-Shikh	19.40 ^e	0.330 ^g	0.900 ^a	74.31 ^b	5.05 ^{cd}	73.50 ^c	6.8 ^b	31.55 ^e	18.23	18.25	3.72 ^c
Abis	17.00 ⁱ	0.571 ^d	0.311 ⁱ	78.17 ^{ab}	3.95 ^{cd}	39.67 ^j	6.3 ^b	26.03 ^f	14.83	11.65	3.87 ^{bc}
Al-Tarh	19.00 ^f	0.415 ^f	0.436 ^{ef}	75.52 ^b	1.33 ^f	83.53 ^a	6.6 ^b	32.48 ^e	18.63	18.89	3.67 ^{cd}
Kom-Esho	19.40 ^e	0.884 ^a	0.423 ^f	74.42 ^b	4.89 ^{cd}	64.52 ^d	8.4 ^{ab}	39.67 ^{cd}	23.83	22.63	3.85 ^{bc}
Dabbor	16.20 ^j	0.099 ⁱ	0.368 ^h	73.82 ^b	9.51 ^b	47.90 ^g	1.8 ^c	36.83 ^d	8.00	28.83	3.47 ^d
El-Temsah	16.00 ^k	0.120 ^k	0.522 ^c	62.60 ^d	15.23 ^a	46.41 ^h	2.1 ^c	35.83 ^d	7.17	28.67	3.45 ^d
Standards											
Egyptian standard	20 ^c	0.5 ^c	0.4 ^g	70.00 ^c	5.00 ^{cd}	40 ⁱ	-	-	-	-	4 ^b
Codex standard	21 ^a	0.5 ^c	0.6 ^b	65.00 ^d	5.00 ^{cd}	40 ⁱ	3 ^c	50 ^a	-	-	-
Gulf standard	21 ^a	0.5 ^c	0.6 ^b	65.00 ^d	5.00 ^{cd}	80 ^b	3 ^c	40 ^{cd}	-	-	5.6 ^a
Saudi standard	21 ^a	0.5 ^c	0.6 ^b	65.00 ^d	5.00 ^{cd}	80 ^b	3 ^c	40 ^{cd}	-	-	-

- HMF = Hydroxymethylfurfural (mg/kg). DN = Diastase number according to (Gothe-Scale number).
- Means at each column followed by the same letter (s) are not significantly different at p=0.05

honey of Al-Temsah. Their reducing sugar content were 60.23, 61.36, 63.51, 60.09 and 62.60 %, respectively.

So, it is recommended to modify the percentage of the reducing sugars in the Egyptian honey standard to be 65 % instead of 70 % to agree with the lower limit of the other international standards.

Reducing sugar content was also reported to be between 75.6 – 81.9 in Bosnia (Mulalic *et al.*, 1985) and 54.26 – 71.72 % (Ivanov, 1997). This was also concurrent with Bogdanov (1999) who suggested new quality criteria which is a minimum content for the sum of fructose and glucose to be 60 g per 100 g of all blossom honey.

Mean sugar content of floral honey was 77.0 % and in honeydew 70.0 % (Bogdanov and Baumann, 1988). Mateo and Bosch-Reig (1997) have also reported that different floral sources could lead to different sugar content as in the sunflower, heather and eucalyptus honeys to be 92.9, 83.3 and 75.0 %, respectively.

5- Apparent sucrose content:

All international laboratories estimate the total disaccharides as apparent sucrose percentage. The percentage of other disaccharides is mainly maltose of about 7 % while sucrose is about 1.5 % for the citrus and clover honey. So, the percentage of the sucrose has to be modified to become 10 % for citrus and clover honey in the Egyptian honey standard instead of 5 %, while the maximum level of 5 % maintains as it is for the cotton honey.

Regarding apparent sucrose content, data showed that honey samples may be divided into two divisions. The first division included the honey samples accepted by the international standards. This division may be subdivided into two groups. The first group would include honey samples which have an apparent maximum sucrose level of 10 % for the citrus and clover honey and 5 % for the cotton honey which agree with the Codex (1998), Gulf (1992) and Saudi (1990). These samples were 10 citrus honey samples, 9 clover honey and 8 cotton honey. The second group included honey samples having an apparent sucrose level under 5 %, which is in harmony with the Egyptian, and subsequently international honey standards. These honey samples were 6 of citrus honey, 8 of cotton honey and 2 of clover honey.

The second division included honey samples that did not agree with all international standards. These honey samples were 2 of citrus honey samples (Abis 17.28 %, and Magdy 16.38 %), 3 of clover honey samples (Al-Abaadia 15.14 %, Al-Tabia 12.57 % and Miser Polan 16.40 %) and 4 of cotton honey (Baslacon 5.84 %, Ezbit El-Shikh, 5.05 %, Dabbor 9.51 % and Al-Temsah 15.23 %).

In Europe, the sucrose limit in honeys reached 5 % in Bosnia and Hersegovina (Mulalic *et al.*, 1985). Honey collected from botanical sources was estimated to have about 0.0 – 3.99 % sucrose (Ivanov, 1997).

6- Hydroxymethylfurfural level:

Regarding Hydroxymethylfurfural (HMF) level, data in Tables 1, 2 and 3 showed that samples can be divided into three groups. The first group included samples which were accepted by the four honey standards. These samples were citrus honey of Kom-Hamada, Damanhour and El-Nahal; clover honey of Ezbit El-Shikh, Baslacon and Abis, Miser Polan and cotton honey Abis, Baslacon and Maatuok with HMF values of 33.23, 19.46, 35.93, 16.17, 39.37, 38.32, 24.70, 39.67, 33.53 and 30.24 mg / kg, respectively. The second group included samples not in agreement with the Codex and Egyptian standards, but accepted from the Gulf and Saudi standards. In other words, the second group has values of HMF between 40 and 80 mg / kg. These samples were 8 of the cotton honey, 6 of the clover honey and 7 of the citrus honey where the lowest value was 40.26 mg / kg in El-Monofia clover honey and the highest was 76.96 mg / kg in Edfina citrus honey.

The third group included honey samples with high levels of HMF being more than 80 mg / kg of HMF and subsequently not in agreement with all standards. These samples were Kafr El-Dawar citrus honey (88.62 mg / kg), Al-Tarh citrus honey (85.18 mg / kg), Al-Tarh cotton honey (83.53 mg / kg) and Al-Abaadia clover honey (81.29 mg / kg).

So, honey should be exposed to the minimum heat during storage and transportation (Wix, 1983). In French honey, it was found that 16 of 23 samples have exceeded the minimum HMF threshold which is 40 mg / kg (Bricage, 1989).

7- Diastase activity:

As for the diastase number (DN), honey samples could be divided into two groups. The first group included accepted honey samples by the all international honey standards (DN value not less than 3). These samples were 11 of citrus honey samples, 10 of clover honey samples and 10 of cotton honey samples with the highest value being 9.5 in Damanhour citrus and the lowest is 3.4 in Magdy commercial brand.

The second group included honey samples that are not in agreement with all international honey standards. These samples were Dabor cotton honey, El-Temsah cotton honey and Miser Polan Clover honey. These samples have DN of 1.8, 2.1 and 1.4, respectively.

It is worth noting that, the diastase number is not among the criteria of the Egyptian standard. The use of diastase number as a basis for measuring honey quality was discussed by White (1992), while the effect of heating on the reduction of DN was reported by Skowronek *et al.* (1994). Huidobora *et al.* (1995) have reported a DN (Gothe scale) value above 19 for multifloral honeys

8- Acidity:

The international standards differs in the expression of honey acidity, some expresses it as total acidity while others as pH.

a. Total Acidity: According to this criterion, honey samples could be divided into two groups. The first group includes honey samples accepted by international standards having acidity content less than 40 meq / kg. These samples are 12 citrus honey, 8 clover honey and 9 cotton honey with highest value of 39.67 meq / kg in Kom-Esho cotton honey, while the lowest value was 19.85 meq / kg in Sidy-Ghazy cotton honey also.

The second group included honey samples that have relatively high total acidity values exceeding the Saudi (1990) and Gulf (1992) limits ranging from 40 to 50 meq / kg. So, they were not in agreement with the Saudi and Gulf standards but accepted by the Codex (1998). These samples were of clover honey from Abis (44.00), El-Monofia (47.27) and Khorshid (40.83) and cotton honey of Baslacon (46.65), Edco (41.67) and Al-Sabhia (45.35).

b. pH: This is a logarithmic scale representing the concentrations of hydrogen ion in solution. All analyzed honey samples are accepted by the Gulf (1992) and Egyptian (1990) honey standards and have pH level less than 4.

The amount of D-gluconic acid was determined enzymatically by Mato *et al.* (1997) and was found to be around 7.37 g / kg while other acids were identified by Nozal *et al.* (1998) using HPLC where they found that pyruvic, citric, formic, succinic, lactic and propionic may contribute to honey acidity.

9- Pollen identification:

Pollen grains in honey samples mounted on microscopic slides were compared with those collected from the flowers of the main sources (citrus, clover and cotton) as a key for pollen identification according to Eredtman (1954).

The data in Table 4 showed that pollen grains extracted from honey samples and examined under the microscope could be divided into five groups.

a. Citrus honey samples:

This group of honey samples had a majority of pollen grains that belong to citrus pollen. These honey samples were from Al-Tarh (55 %), Baslacon (60 %), Danamhour (65 %), Kom-Hamada (73 %), Edfina (80 %), Khat Wistany (74 %), Al- Maamora (82 %), Khorshid (87 %) and Kafr El-Dawar (92 %).

b. Clover honey samples:

This group of honey samples had a majority of pollen grains that belong to clover pollen. These honey samples were from Kafr El-Dawar (57 %), Abis (60 %), Baslacon (70 %), El-Nozha (71 %), Damanhor (77 %), Khorshid (78 %), El-Monofia (82 %) and Azbit El-Shik (87 %).

c. Cotton honey samples:

This group of honey samples had a majority of pollen grains that belong to cotton pollen. These honey samples were from Al-Sabhia (5 %), Edco (15 %), Baslacon (20 %), Al Tarh (30 %), Azbit El-Shik (32 %), Abbis (33 %), Maatouk (35 %), Komosho (36 %), Sidy Ghazy (40 %), and Abou Homous (42 %).

Table (4): Pollen grains percentages (citrus, clover and cotton) determined separately in honey samples collected from citrus, clover and cotton foraging areas.

Honey samples	% Pollen grains		
	Citrus	Clover	Cotton
Kafr El Dawar	92	57	-
Khorshid	87	78	-
Al ma'mora El balad	82	-	-
Edfina	80	-	-
Khat Westany	74	-	-
Kwom Hamada	73	-	-
Damanhor	65	77	-
Abis	62	60	33
Baslacione	60	70	20
Al Tarh	55	-	30
El Nahal	0	-	-
Magdy	0	-	-
Ezbit El Shikh	-	87	32
El Monofia	-	82	-
El Nozha	-	71	-
Al Aba'dia	-	0	-
Al Tabia	-	0	-
Misr polan	-	0	-
Abo Homous	-	-	42
Sidy Ghazy	-	-	40
Komosho	-	-	36
Ma'tuok	-	-	35
Edco	-	-	15
Al Sabhia	-	-	5
Dabour	-	-	0
El Temsah	-	-	0

(-) Not determined

(0) No pollen grains of the corresponding species.

d. Mixed honey samples:

This group included honey sample that contained pollen from different flower sources. This kind of honey was represented by only one sample, Al-Sabbia, which had 10 % of cotton pollen, 42 % of clover pollen, 40 % of citrus pollen and 8 % of other types of pollen.

d. Honey without pollen grains:

This group included honey samples free of pollen grains. These samples were of citrus honey of Abbis and Magdy, of clover honey of Al-Abaadia, Al-Tabia and Miser Polan, and of cotton honey samples of Dabor and El-Temsah.

Several authors have identified several types of pollen in wild, unifloral and multifloral honeys. Gomez-Ferreras (1990) reported up to 80 pollen types. Pollen grains from 17 families were also reported by Sorkun *et al.* (1989) while Carvalho *et al.* (2001) found 28 pollen types dominated by *Eucalyptus* spp.

In conclusion, it is recommended that the Egyptian honey standard of 1990 should be modified as in Table (5). The lower limit of reducing sugar must be changed to 65 % instead of 70. The maximum limit of HMF content

Table (5): Suggested modifications for the Egyptian honey standards.

Criteria	Present Egyptian standard (1990)	Suggested Egyptian honey standard
Moisture content (%)	≤20	≤20
Water insoluble solids (%)	≤0.5	≤0.5
Ash content (%)	≤0.4	≤0.6
Reducing sugars (%)	≥70	≤65
Apparent sucrose content (%)	≤5	≤10 for clover and citrus honey, ≤5 for cotton honey
Hydroxymethylfurfural content (HMF) mg/kg	≤40 mg/kg	≤80
Diastase number	--	≥3
PH	≤4	≤4

must be increased to 80 mg / kg instead of 40 mg / kg as Gulf and Saudi standards since the Arab countries import large amount of Egyptian honey. The sucrose content has to be modified to apparent sucrose content with a maximum of 10 %. The diastase activity (DN) should be included in the Egyptian standard with a minimum limit of 3.0. Also, the maximum limit of ash content should be increased to 0.6 instead of 0.4 %.

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دراسات فيزيائية و كيميائية على خواص عسل النحل المصري مقارنة بالمواصفات القياسية للعسل

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تهدف الدراسة الى تقييم المواصفات القياسية المصرية لعسل النحل لسنة ١٩٩٠ وقد تم جمع ٣٥ عينة عسل نحل مفروز بمعدل ثلاث مكررات لكل عينة من محافظتي الإسكندرية والبحيرة وقد تضمنت عينات عسل النحل المفروز ثلاثة أنواع هي عسل الموالح ، عسل البرسيم و عسل القطن طبقاً لمواسم القطف. وكذلك تضمن البحث بعض العينات التجارية. وقد تم تحليل هذه العينات طبقاً لطرق التحليل الأساسية لجمعية الكيمائيين التحليليين لسنة. ولقد تم في جميع العينات تحديد كل من السكروز والسكريات المختزلة (الجلوكوز والفركتوز) والمحتوى الرطوبى ومعامل الانكسار والمواد الصلبة الغير ذائبة فى الماء والرماد والحموضة الحرة واللاكتون والحموضة الكلية ونشاط إنزيم الدياستيز والمحتوى من الهيدروكسى ميثيل فيرفيورال. طبقاً للمواصفة القياسية العالمية لعسل النحل (الكودكس) لسنة ١٩٩٨. كما وتم أيضاً دراسة محتوى العسل من أنواع حبوب اللقاح. وقد قورنت النتائج مع المواصفات القياسية السعودية لعسل النحل لسنة ١٩٩٠ والمواصفة القياسية لعسل النحل لدول مجلس التعاون الخليجى لسنة ١٩٩٢ والمواصفة القياسية المصرية لعسل النحل لسنة ١٩٩٠.

ومن هذه الدراسة يمكن التوصية بأنه يجب تعديل المواصفة القياسية المصرية لعسل النحل لتتواءم مع المواصفات العالمية الأخرى وذلك فى البنود الآتية :

- ١ - الحد الأعلى للسكروز الظاهر لعسل الموالح والبرسيم لا يزيد عن ١٠% أما فى حالة عسل القطن فلا يزيد عن ٥% .
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- ٤ - الحد الأعلى للهيدروكسى ميثيل فيرفيورال ٨٠ مجم / كجم .
- ٥ - يجب أن تتضمن معيار نشاط الدياستيز بحد أدنى ٣ وحدات على مقياس جوث.