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Physicochemical Characteristics of Citrus and Clover Honey Collected from Different Egyptian Regions

Ghada f. Badran[1], Soad T. Khir[2], Mohamed R. Badr[1] and Mohamed B. Atta[1]

[1] Food Science and Technology Department, Faculty of Agriculture, Tanta University, Egypt
2 Food Technology and Dairy Department, Faculty of Agriculture, Damanhour Univeristy, Egypt.

Corresponding Author: gfwad3977@gmail.com
Mohamed_badr@agr.tanta.edu.eg ,bassimatta@yahoo.

J.Sust.Agri.Env.Sci. (JSAES)

ABSTRACT:
Honey is a natural product that is normally used for its sweetness, gives energy, and has health benefits. Nowadays, honey adulteration has widely spread, as it has lost much of its nutritional value and poses a risk to the public health of consumers. So, its physicochemical characteristics were affected according to the degree of adulteration. Therefore, this study aimed to evaluate the quality of Egyptian honey in the local market by comparing two types of honey (citrus and alfalfa) that were collected from apiaries in different regions during the seasons 2021-2022.

The results revealed that the quality of the honey, including its physicochemical properties, enzyme activity, and heavy metals content differed according to the geographical and environmental botanical origins. The pH of honey also varies according to the variations of the season. Where the pH of most samples ranges from (3.40 to 6.10) matching the standard acidity limit. The citrus honey was the most acidic one (pH = 3.33). The concentration of sucrose in the commercial citrus honey collected from Qalyubia (12.34%) was higher than that of clover honey collected from Upper Egypt (9.75%). It is interesting to note that some of the commercial samples contain hydroxymethylfurfural has lower proline and lower diastase activities which indicate the honey is heated, stored in bad conditions or adulterated.

Keywords:
Honey bee, physicochemical characteristics, Hydroxymethylfural (HMF), Adulteration

1. INTRODUCTION

Honey is a complex product that is easy to digest and absorb, it is produced from nectar, and nectar is a sugary liquid from flowers, honey is produced by the action of enzymes produced by bees, for example, diastase, invertase and glucose oxidase **(Can *et al.*, 2015).**.

Honey is a wonderful source of energy, because it is composed of 80% carbohydrates, the percentage of glucose, fructose and sucrose that it contains is about (35%, 40% and 45%), respectively, and it contains 20% water, In addition, it contains more than 200 component parts, including phenolic compounds, organic acids, vitamins, minerals, enzymes and amino acids **(Kahraman *et al.*, 2010).**

The honey bee of the family (*Apis mellifera* L) is one of the most important organisms that are affected by environmental conditions, and because they have a great ability to sense environmental changes, they are considered a biological indicator of many toxic environmental factors, that exist in nature **(Celli and Maccagnani, 2003).**

Bees are always in contact with soil, air and water, and this reflects the concentration of minerals in honey, and the amount present in the surrounding area. **(Atrouse *et al.*, 2004)**, the ash content and acidity of honey are related to its electrical conductivity, showing the presence of ions, organic acids, and proteins **(Dasilva *et al.*, 2016).**

The most important quality factor for honey is its moisture level, because the fermentation rate, shelf life and processing properties are largely determined according to its moisture content **(Gebremedhin *et al.*, 2013).**

Because honey is hygroscopic, the amount of water in it varies depending on the climatic conditions, the strength of the bee colony, and the plant origin of the honey, as well as the humidity of the outside air during the honey sorting process, temperature, treatment, and storage **(Stamatovska *et al.*, 2018).** The viscosity of honey depends on several factors, the most important of which are moisture content, chemical composition and temperature **(Kang *et al.*, 2008)**, the composition of honey varies, according to its botanical and geographical origin **(Nguyen *et al.*, 2019),** it is known that honey is rich in trace elements such as cadmium, nickel and lead, known as toxic metals, in addition to other essential minerals such as zinc, copper, manganese and chromium, which are important for human health and growth within specific restrictions. **(Hernández *et al.*, 2005),** however, excessive use of these ingredients can lead to chronic toxicity **(Pohl, 2009)**

Heavy metals are chemical elements, characterized by a high relative density, and are found in waste collection areas, industrial complexes and places where agricultural chemicals are extensively used **(Shaban *et al.*, 2016),** contamination of honey with heavy metals poses a danger to the human who consumes it, especially when the pollution accumulates to toxic levels. Moreover, the sources of contamination from minerals vary from external sources such as an industrial source or incorrect methods of processing honey, or they could be due to agrochemicals and pesticides. **(Ru *et al.*, 2013).**

Hydroxymethylfurfural (HMF) is a water-soluble heterocyclic organic compound derived from sugars, HMF is a decomposition product of fructose (dehydration of fructose), which is naturally found in a trace amount and its concentration increases with honey storage and extended heating, the concentration of HMF increases when honey undergoes short-term heat treatment at a high temperature to reduce viscosity and prevent crystallization **(Subramanian *et al.*, 2007).**

HMF is produced in an acidic environment, at low temperatures, and at high temperatures, and prolonged precipitation leads to a significant increase in its concentration **(Shapla *et al.*, 2018).**

Iron (Fe) is important for the formation of red blood cells, and it can mediate the transfer of electrons to catalyze enzymatic reactions, but it is potentially dangerous because it can catalyze the conversion of hydrogen peroxide into free radicalsaccording to **Zugravu *et al.* (2009).**

Heavy metals such as lead and cadmium can damage the environment (contamination of soil and water, accumulation in plants); they also pose significant health risks to humans (kidney disease, lung damage, nervous system failure), estimates revealed that the majority of honey samples analyzed were not free of heavy metals, although their amounts were well above the permissible limits, unlike other results, the physicochemical composition of honey, which is strongly influenced by geographical area, floral supply, climate, environmental conditions, and the treatment of beekeepers, should be examined **(Joseph *et al.*, 2007; Kaškonien *et al.*, 2010).**

For many years, there has been a great deal of interest in sourcing the honey plant, there are several reasons for wanting to determine the floral origin of honey, including quality control in marketing, and regulatory concerns about the honey's country of origin **(Molan, 1998).**

The geographical and botanical origins of honey have affected its quality and physical and chemical properties **(Taha *et al.*, 2016; Khaled and Abdel-Hameed, 2020).**

The main objective of this study was to determine the safety of producing different types of honey varieties in Egypt (6 samples), where the increasing environmental pollution and the spread of diseases have led to a decrease in the number of honey bees in the world. it means that honey is becoming an increasingly scarce commodity, so the adulteration of honey is on the rise.

2. MATERIALS AND METHODS

Honey samples collection:

Six samples of honey were collected in different regions of Egypt during the seasons of the year 2021 according to floral sources, Citrus, Clover, Citrus honey samples were collected in April, and clover honey samples were collected in June, Citrus honey is produced by honey bee workers from the nectar of the flowers of different citrus species at the beginning of spring until the end of the flowering period of citrus species (1st season of yield). Clover honey is being produced by honey bee workers in the period of May-June during the flowering of clover (2nd season).

Six different samples of citrus honey and clover honey were collected from different places as follows:

- Citrus honey samples were (A- B- C), samples (A) were collected from Beheira governorate, (B) samples were collected from Kafr El-Zayat near the road, and (C) samples were purchased from Qalyubia governorate **(Commercial honey).**
- Clover honey samples were (D- E- F), honey samples (D) were collected from Tanta, honey samples (E) were collected from El-Mehala near the road, and honey samples (F) were purchased from Upper Egypt **(Commercial honey).**

Environmental conditions during sample analysis:

- The temperature was: 25°C.
- The humidity was equivalent to: (38%) RH.

Methods description:

1- The pH was measured with a pH meter (Boeco, Germany) pH = 4,7 and 10 when calibrated with buffers.
2- Acidity was determined by the accumulated equivalence point titration, according to **Bogdanov *et al.* (2002)**, the results were presented in (meq/kg)

3- Electrical conductivity of honey was measured using (Conductivity meter, five easy, mettler, Switzerland). The determination was achieved at a temperature of (20°C) for (20%) of honey weight in volume solution in water, where (20%) refers to honey dry matter, the method of measurement was determined by the methods of the **International Honey Commission (2009)**.

4- Viscosity of honey was measured in centipoise using FUNGILAB viscometer. The sample was placed in a beaker and the spindle was inserted into the sample. Measurements were taken at 40°C.

5- The insoluble matter is collected in a crucible with a predetermined pore size, and the dried residue is weighed after being cleaned free of soluble material.

6- The water content was determined using a digital refractometer (Boeco, Germany), according to **International Honey Commission, (2009)**. The formula TS (%) = 100 - M was used to calculate the total solids expressed as a percentage. Where: TS = Total Solids (%); M = sample moisture content (%).

7- Specific gravity: Using a pycnometer, the gravimetric method was used to determine the specific gravity of raw honey, and the values were calculated by dividing the mass of the pycnometer (50 ml bottle filled with honey) by the mass of the same bottle filled with distilled water.

8- The reducing sugars (%) and sucrose content (%) were analyzed following the **Codex Alimentarius Committee on the Codex Standard for Honey (2001)**.

9- Hydroxy methyl furfural (HMF) was determined by a spectrophotometer after white method.

10- Diastase activity was determined after shading to obtain a D.N (Gothe unit). One unit is defined as the amount of enzyme that will convert 0.01 g of starch to the prescribed end-point in 1 h at 40°C, as per the test conditions used by the **International Honey Commission (2009).**

11- Proline is defined as the color developed with ninhydrin compared to the praline standard and expressed as a percentage of the mass of honey in mg/kg. Proline content was determined using the harmonized methods of the **International Honey Committee (2009)**.

12- Determination of the proline instrument used (UV/VIS) spectrophotometry, Genway, England, wavelength (510 nm).

13- Determination of hydrogen peroxide: in the presence of peroxidase (HRP), (H_2O_2) reacts with 3.5-dichloro-2•hydroxy-benzenesulfonic (DHBS) acid and 4-amjnophenazone (AAP) to form a choromphore 2H202 + DHBS + AAP – HRP-. QUINONEIMINE DYE + 4H20 according to **Brudzynski *et al.* (2011).**

14- Determination of ash and heavy metals in honey: The ash content was determined using amuffle furnace at 550-600°C until a constant weight was reached according to the methods described by **AOAC. (2000)**. The concentrations of minerals iron (Fe), zinc (Zn), lead (Pb), cadmium (Cd), were determined by an atomic absorption spectrophotometer (AAS Model SP9) Heavy metal concentrations in the sample were calculated in mg/kg according to **Osman *et al.* (2007)**

15- **Statistical Analysis:** The statistical analysis was carried out using one-way (ANOVA) using (SPSS, ver. 25) (IBM Corp. Released 2013). The data was treated as a complete randomization design according to **Steel *et al.* (1997)**. Multiple comparisons were made using the Duncun test. The level of significance was set at < 0.05.

3. RESULTS AND DISCUSSION

.**Physical properties of citrus honey and clover honey:**

The data in Table (1) shows the pH values for six honey samples. Where the pH value of the samples showed that they were acidic, the pH ranged from (3.33: 4.14), and within the standard limit (pH of 3.40 to 6.10) **(CAC, 2001)** Among all the studied types of honey, citrus honey (A) was the most acidic (pH = 3.33), the rest of the samples are arranged as follows: E (pH = 3.72), D (pH = 3.96), F (pH = 3.98), C (pH = 4.10), B (pH = 4.14).

The pH of honey varies according to the different growing seasons and geographical regions, these results agree with what was previously reported with Indian, Algerian, Brazilian, Spanish and Turkish honey (between pH 3.49 &4.70) **(Saxena *et al.*, 2010; Rebial *et al.*,2015).**

There is a significant difference between citrus honey (C) from Qalubia, (pH4.10±0.01) and citrus honey (A) from Buhaira (pH 3.33±0.03), this difference may be due to deterioration of honey during storage, or the possibility of adulteration of the sample, by adding syrup sugar.

Total acidity:

Total acidity differed significantly among the tested species. Honey samples characterized by the lowest average total acidity were citrus honey (c) from Qalyubia (24.41±0.29), compared with other honey samples. The total acidity of the honey ranged from 24.41±0.29 (citrus c) to(33.57±0.21) meq/kg (clover E). Differences between the findings obtained from several studies and our findings may be due to differences in geographical conditions, harvesting procedures and storage conditions , and the possibility of heating or adulteration with sugar.

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Table (1): Mean values of some physical characteristics of citrus and clover honey (mean±SE).

Type	Place	pH	Free acidity (meq/kg)	Lactone (meq/kg)	Total acidity (meq/kg)	Viscosity CP	EC* (MS/cm)	Specific gravity
Citrus	(A) Buhaira Governorate	3.33 ±0.03[d]	27.15 ±0.02[a]	4.29 ±0.04[e]	31.44 ±0.06[b]	48305 ±1.0[b]	0.163 ±0.018[f]	1.41 ±0.00[b]
	(B) Kafr El-Zayat	4.14 ±0.01[a]	11.97 ±0.14[f]	16.31 ±0.04[a]	28.28 ±0.17[c]	48212 ±2.0[d]	1.000 ±0.006[a]	1.41 ±0.00[b]
	(C) Qalyubia Governorate	4.11 ±0.01[a]	14.03 ±0.04[e]	10.38 ±0.25[d]	24.41 ±0.29[e]	48162 ±0.0[e]	0.903 ±0.015[b]	1.40 ±0.00[c]
Clover	(D) Tanta	3.96 ±0.02[b]	16.33 ±0.05[d]	10.37 ±0.12[d]	26.70 ±0.16[d]	48337 ±1.0[a]	0.717 ±0.019[d]	1.42 ±0.00[a]
	(E) El-Mehala	3.72 ±0.02[c]	18.16 ±0.17[b]	15.41 ±0.04[b]	33.57 ±0.21[a]	48221 ±1.0[c]	0.427 ±0.02[e]	1.40 ±0.00[c]
	(F) Upper Egypt	3.98 ±0.02[b]	17.42 ±0.19[c]	11.33 ±0.07[c]	28.75 ±0.15[c]	48201 ±1.0[d]	0.770 ±0.032[c]	1.41 ±0.00[b]

(a, b & c): There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter,

* EC: (ms/cm) Electrical conductivity, Free acidity, lactone and total acidity (meq/kg): mill equivalent of free acidity per kilogram of honey, viscosity were expressed in centipoise(CP)

Viscosity ratio:

The highest viscosity value was (48337±1.0) at (40°C) observed in clover honey(D) collected from Tanta , while the lowest was (48162±0.0) at 40°C in citrus honey (C) from Qalyubia, (Table 1). The various differences between the viscosity of honey can be attributed to the moisture content, sugars, and protein concentrations, which vary according to the different geographic and floral origins of each honey. **(Nayik *et al.*, 2018),** The difference in viscosity also indicates the possibility of adulteration in honey.

Electrical Conductivity (EC):

As shown in Table No (1), the highest (EC) was in citrus honey (B) from Kafr El-Zayat: (1.000±0.006), citrus honey (C) from Qalyubia (0.903±0.015), clover honey (F) from Upper Egypt (0.770±0.032), clover honey (D) from Tanta (0.717±0.019) and clover honey (E) from El-Mehala (0.427±0.02), respectively. The conductivity of citrus honey(B) from Kafr El-Zayat was found to be greater than other types due to its higher mineral content. **(Shehata *et al.*, 2018).** The observed significant variation in electrical conductivity in studied honey samples may be attributed to differences in degree of maturity, soil composition, climatic factors and the possibility of adulteration by adding sugar.

Specific gravity:

It depends on the water content of honey**,** the specific gravity of the honey samples that were examined ranged from (1.40: 1.42) Table No. (1) **(Essa *et al.*, 2010**). It was determined that the specific gravity of the different types of clover honey ranged (1.40: 1.45).

Chemical properties of citrus and clover honey:

Moisture content:

The data in Table No (2) showed that the moisture percentages in the honey samples ranged from16.14±0.02 to 22.58±0.03 The moisture content of honey varies from year to year depending on environmental circumstances and the beekeeper's manipulation throughout the harvest season, high moisture content in honey may accelerate crystallization **(Acquarone *et al.*, 2007).**

The moisture content values also comply with the proposed level of **CAC (2001)** for bee honey moisture content which stated that it shouldn't be more than 21%, but the moisture content was highest in citrus honey (c) from Qalyubia Governorate (22.58±0.03). These variations in the moisture content of honey under study could may be attributed to temperature and high relative humidity of sampling location, method of honey extraction, and storage conditions. High humidity indicates that honey may have been adulterated by mixing it with sugar syrup (processed honey).

HMF:

According to the current study, the highest concentrations were found in clover honey (F) from Upper Egypt, which is 82.67±0.01, and the lowest concentrations were found in citrus honey (A) from Buhaira Governorate, which is 3.48±0.02.

The reasons for the high level of (HMF).due to, temperature, heating period, storage conditions, type and acidity of honey**,** therefore, it is used as an indicator for heating honey at high temperature, poor storage conditions or adulteration, by adding invert sugar (syrup) and because HMF can be produced by heating sugars **(Belay *et al.*,2017; Ahmed *et al.*, 2014 and Sakač *et al.*, 2019)**.

Total proline:

The results showed a high percentage of proline in citrus honey (A) from Beheira Governorate, it was (293.83±0.76), followed by citrus honey (B) from Kafr El-Zayat (279.57±0.07), then clover honey (D) from

Tanta (262.23±0.09), and clover honey (E) from El-Mehala (153.27±0.19), while the lowest content of proline was in clover honey (F) from Upper Egypt (54.8±0.01) and citrus honey (C) from Qalyubia (117.23±0.03).

The variation in proline content in the honey samples is attributed to the fact that the proline concentration was reduced to less than 180 mg/kg when invert sugar and sucrose syrup were added. Since the amount of proline in honey constantly decreases during storage, proline may be an indicator of the maturity of honey, according to **Czipa *et al.*(2012).** Keep in mind, there is a wide variation in the total proline content depending on the type of honey **(Al-Farsi *et al.*, 2018).**

The level of proline in honey is an indicator of its maturity, and it is also used to detect adulteration with sugar, its minimum in natural honey is 180 mg/kg of honey, according to the standard specifications of honey**.**

Total Solids:

The total solids (%) in the studied honeys were(83.86±0.02, 82.32±0.04, 81.78±0.02, 81.05±0.03, 80.56±0.20 and 74.42±0.03) for samples(A, B, C, D and E,)respectively in Table No (2), in this respect, other than a sample of citrus honey from Qalyubia (C) where the percentage of total solids was low, (77.42±0.03), due to the high moisture content of honey, it was(22.58±0.03), this sample is subject to fermentation during storage and a short shelf life. The results were in agreement with **Rebial *et al.* (2015)**, who reported that, the total solids were within acceptable international range of 80: 90%.

In Soluble Matter:

According to the data in Table No (2), the results showed that the highest content of dissolved substances (%) was found in clover honey (F) from Upper Egypt (2.407±0.032), citrus honey (C) from Qalyubia (1.607±0.012), clover honey (E) from El-Mehala (1.313±0.038) and citrus honey (B) from Kafr El-Zayat(0.930±0.026). These results are above the maximum allowable by the standard specification of honey which should not exceed 0.5 g/100 g.

While the low samples were citrus honey (A) from Buhaira Governorate (0.219±0.003), and clover honey (D) from Tanta (0.370±0.038), these results were in accordance with the standard specification for honey. The high percentage of insoluble solids in water is due to the presence of pollen, propolis and beeswax residues in the samples.

Ash:

The highest percentage of ash (%) was in the clover honey (F) from Upper Egypt (0.41±0.01), while the lowest percentage was in citrus honey (A) from Buhaira Governorate (0.09±0.00).

The difference in the percentage of ash is due to the plant origin of honey, where ash is a quality factor to determine the plant origin of honey **(Felsner *et al.*, 2004),** and this suggests that the honey from Upper Egypt contains high levels of impurities and unwanted particles which make ash content higher and that the honey was also sourced from the different species and geographical regions. Also, harvesting honey under different conditions, processing it under different conditions, adulterating with different adulterants materials raises the ash contents,also because of the high percentage of minerals in honey , where the ash content is related to the amount of minerals in the honey.

Sucrose content and reducing sugars in honey:

All samples were in conformity with the standard specifications, as the results were as follows**:** citrus (A) Buhaira sucrose (3.63±0.02), reducing sugars (69.14±0.01) citrus (B) Kafr El-Zayat Sucrose (6.73±0.02), reducing sugars (66.74±0.02) clover (D) Tanta sucrose (3.51±0.01), reducing sugars (67.85±0.03).

There is a significant difference between the samples, a high percentage of sucrose was observed in citrus honey (C) from Qalyubia Governorate (12.34±0.02), while the reducing sugars was (61.02±0.00), followed by clover honey (F) from Upper Egypt, the proportion of sucrose was (9.75±0.03) and its reducing sugars was (59.99±0.02) and clover honey (E) from El-Mehala sucrose (7.13±0.01) and reducing sugars (67.97±2.00). by comparing the results with the standard specification for honey. It was found that the sucrose content was above a maximum of 10%, while the reducing sugars were close to the minimum 60%.

The increased concentration of sucrose in honey may be due to the early harvest in which sucrose did not convert to fructose and glucose **(Azeredo *et al.*, 2003**), and the possibility of adding sucrose syrup to honey.

Table (2): Mean values of some chemical properties of citrus and clover honey.

Type	Place	Moisture (%)	T.S. (%)	In soluble matter (%)	Ash (%)	Sucrose (%)	Reducing sugar (%)	HMF (mg/kg)	Total proline (mg/kg)
Citrus	(A) Buhaira Governorate	17.68 ±0.04[e]	82.32 ±0.04[b]	0.219 ±0.003[f]	0.09 ±0.00[f]	3.63 ±0.02[e]	69.14 ±0.01[a]	3.48 ±0.02[e]	293.83 ±0.76[a]
	(B) Kafr El-Zayat	18.95 ±0.03[b]	81.05 ±0.03[d]	0.930 ±0.026[d]	0.32 ±0.00[c]	6.73 ±0.02[d]	66.74 ±0.02[b]	3.52 ±0.02[e]	279.57 ±0.07[b]
	(C) Qalyubia Governorate	22.58 ±0.03[a]	77.42 ±0.03[f]	1.607 ±0.012[b]	0.12 ±0.01[e]	12.34 ±0.02[a]	61.02 ±0.00 [c]	77.95 ±0.02[b]	117.23 ±0.03[e]
Clover	(D) Tanta	16.14 ±0.02[f]	83.86 ±0.02[a]	0.370 ±0.038[e]	0.26 ±0.00[d]	3.51 ±0.01[f]	67.85 ±0.03[ab]	15.51 ±0.29[d]	262.23 ±0.09[c]
	(E) El-Mehala	18.22 ±0.02[c]	81.78 ±0.02[c]	1.313 ±0.038[c]	0.36 ±0.00[b]	7.13 ±0.01[c]	67.97 ±2.00[ab]	41.76 ±0.03[c]	153.27 ±0.19[d]
	(F) Upper Egypt	19.44 ±0.20[d]	80.56 ±0.20[e]	2.407 ±0.032[a]	0.41 ±0.01[a]	9.75 ±0.03[b]	59.99 ±0.02[c]	82.67 ±0.01[a]	54.8 ±0.01[f]

There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

HMF (mg/kg): Hydroxymethylfurfural in milligram per kilogram of honey (T.S %) total solids.

Minerals and Heavy metals content in honey:

Lead content in honey:

Data in Table No (3) clearely showed that, the highest content was found in honey sample clover (E) from El-Mehala (1.025 ±0.007 mg/kg)). The lowest average lead content was classified in the honey samples (A) collected from Beheira Governorate (0.114±0.002 mg/kg). While the results of the other samples were recorded and were as follows: citrus (B) from Kafr El-Zayat (0.828±0.003 mg/kg) citrus (C) from Qalyubia Governorate (0.542±0.005 mg/kg), clover (D) from Tanta (0.421±0.008 mg/kg), and clover (F) from Upper Egypt (0.728±0.009 mg/kg).

The obtained results were compared with the allowed upper limit according to **EUC (2000)** standard (maximum residue limit) (MRL = 1 ppm) It was found that, all samples are not contaminated from the point of view **(EUC, 2000)**, except for the clover (E) where it was (1.025±0.007)mg/kg, and this value is above the permissible limit, according to this specification.

The maximum allowable limit for lead in bee honey has been established according to **CAC (2001)**=(0.1 ppm) according to this specification, these samples are considered non-conforming. There is a significant difference between clover (E) from El-Mehala and citrus (A) from Beheira Governorate. The high level of (Pb) in honey, collected from bee colonies in the region of Kafr El-Zayat, may be due to vicinity of some apiaries to the traffic roads, where large quantities of exhaust cars are generated.

Atmospheric pollutant deposition on plants, may be absorbed together with flower nectar, pollute combs through contaminated bees bodies, or contaminated water and pollen which collected by bees to the hive **(Porrini *et al.*, 2003).**

One easy way for lead to enter our food chain is to spread it on flowering plants **(Fakhimzadeh and Lodenius, 2000).** Polluted pollen will raise the level of metals in honey and other hive products **(Zugravu *et al.*, 2009).** Lead contamination of samples is due to the presence of the apiary near the highway, and may be due to the use of organic fertilizers, pesticides and drugs used to treat bee diseases.

Cadmium content in honey:

The highest average content of cadmium, was estimated as it was found in citrus (B) from Kafr El-Zayat (0.092±0.001mg/kg), and the lowest average cadmium content, was classified in the honey sample (A) collected from Beheira Governorate (0.009±0.001 mg/kg). While the results of the other samples were recorded and were as follows: citrus honey (B) from Kafr El-Zayat (0.092±0.001 mg/kg) citrus honey (C) from Qalubia (0.068±0.0008 mg/kg), clover (D) from Tanta (0.036±0.001 mg/kg), and clover honey (F) from Upper Egypt (0.054±0.003 mg/kg).

The obtained results were compared with the allowed upper limit according to the EUC standard (MRL = 0.1 ppm), it was found that all samples are not contaminated from the (EUC) point of view, **(EUC, 2000)**. It has been observed that a high percentage of cadmium in Kafr El-Zayat citrus honey, (0.092±0.001 mg/kg), and this percentage is close to the maximum allowed in this specification. The maximum allowable limit for cadmium in bee honey has been established according to **CAC (2001)** = (0.05 ppm).

According to this specification, there are samples that are none higher than the maximum limit for this specification, samples are: citrus (B) from Kafr El-Zayat (0.092±0.001 mg/ kg), citrus (C) from Qalubia (0.068±0.000 mg/kg), clover (E) from El-Mehala (0.073±0.000 mg/ kg), and clover (F) Upper Egypt (0.054±0.003 mg/kg). Therefore, it does not conform to this specification. The samples conforming to this standard are citrus (A) from Beheira (0.009± 0.001 mg/kg), clover (D) from Tanta (0.036± 0.001mg/kg). These results are close to the results obtained by (**Hassan *et al.*, 2010)**

There is a significant difference between the samples due to the wrong practices of beekeepers in using smokers in the beehive to keep the bees away from the hive when they are examined, and also due to the lack of cleanliness of the tin containers used for storing honey, and the presence of the apiary near the traffic road is an indication of honey contamination with traces of cadmium. In general Honey in some areas can be a good indicator of cadmium and lead, especially polluted areas that are generally located near traffic roads and indeed the level of lead and cadmium in honey was higher than in isolated areas. On average, the reason for the higher maximum cadmium may be due to the low use of plastic containers for storing honey**(Chandrama *et al.*, 2014)**

Heavy metals present in honey in higher than permissible quantities pose a danger to the human body, due to the potentially harmful effects of pollutants.

The high percentage of heavy metals in honey is due to air and water pollution with toxic heavy metals from transportation emissions, which are then transmitted to the beehives, polluting them and their products. Lead and cadmium can reach honey through the air, while cadmium reaches the soil through water and from it to the plant and reaches the end to the nectar of flowers, and due to the movement of bees through the air, heavy metals can stick to the hairs on the body of the bee and return it to the hive with pollen, or through its absorption of nectar or water contaminated with these elements.

Zinc content in honey:

The data in Table No (3) showed that the highest concentration of zinc was in clover honey (D) from Tanta (2.038±0.016 mg/kg), and the lowest concentration was in citrus honey (A) from Beheira (0.182±0.002 mg/kg). The significant difference in zinc concentration in the samples is due to the difference in the season of collection and the type of pasture plants, in addition to the type of honey **(Al Naggar *et al.*, 2013).**

The high proportion of zinc may be due to the use of fertilizers and agricultural pesticides at the honey collection site. By comparing the results obtained with **CAC (2001)** (Zn = 5ppm), we find that all results are less than the maximum allowed in this standard. Therefore, it complies with these specifications.

Fe content in honey:

The results in Table No (3) showed that the highest concentration of iron (Fe) was in citrus honey (B) from Kafr Elzayat (12.376±0.012 mg/ kg), and the lowest concentration of iron was in clover honey (D) from Tanta (1.189±0.004 mg/kg), the table showed significant values between citrus honey and clover honey.

By comparing the results obtained with **CAC (2001)** (Fe = 20) ppm, we find that all results are less than the maximum allowed in this standard. Therefore, it complies with these specifications. These results are in agreement with the results reported by **Tiwari *et al.* (2016).**

Table (3): Minerals and heavy metals levels in citrus and clover honey (Mean±SE).

Type	Place	Pb (mg/kg)	Cd (mg/kg)	Fe (mg/kg)	Zn (mg/kg)
Citrus	(A) Beheira Governorate	0.114 ±0.002[f]	0.009 ±0.001[f]	3.006 ±0.002[c]	0.183 ±0.001[f]
	(B) Kafr El-Zayat	0.828 ±0.003[b]	0.092 ±0.001[a]	12.376 ±0.012[a]	0.254 ±0.001[d]
	(C) Qalyubia Governorate	0.542 ±0.005[d]	0.068 ±0.000[c]	2.863 ±0.033[d]	0.433 ±0.001[b]
Clover	(D) Tanta	0.421 ±0.008[e]	0.036 ±0.001[e]	1.189 ±0.004[f]	2.038 ±0.009[a]
	(E) El-Mehala	1.025 ±0.007[a]	0.073 ±0.000[b]	4.216 ±0.003[b]	0.376 ±0.000[c]
	(F) Upper Egypt	0.728 ±0.009[c]	0.054 ±0.003[d]	2.662 ±0.017[e]	0.169 ±0.005[e]

There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

Lead (Pb), Cadmium (Cd), Iron (Fe), Zink (Zn) The sample concentrations of heavy metals were calculated in mg/kg.

These results indicate that citrus honey has high iron content. This difference is due to the type of honey and the concentration of this element in the nectar of flowers, in addition to climatic conditions of the areas from which honey was collected **(Shah *et al.*, 2014).** Finally, the variations in the obtained values are connected to the weather conditions, plant type, soil nature, proximity to pollution sources, and beekeeper technique.

Diastase activity in honey:

The data in Table No (4) showed that the highest concentrations of DN as found as clover honey (D) from Tanta (14.06±0.04), citrus honey (A) from Buhaira Governorate (11.36±0.03) and citrus honey (B) from Kafr El-Zayat (5.30±0.01), respectively. The other samples are as found as clover honey (E) from El-Mehala (3.30±0.01), clover honey (F) from Upper Egypt (2.90±0.01e), citrus honey (C) from Qalyubia Governorate (2.81±0.02).

Diastase activity of at least (8 Schade) units overall, and at least (3 Schade) units in honey that are naturally low in the enzyme, according to **CAC (2001)**.

This result corresponds to the standard. The diastase enzyme is present in natural honey but is sensitive to heat, and can indicate a higher product temperature **(Huang *et al.*,2019)**

The enzyme content in honey varies with the age and type of the bee, the type of nectar and the period of collection. Also, the amount of nectar flow and its sugar content, resulting from a highly concentrated nectar flow, reduce the enzyme content as well as the amount of pollen consumed.

The diastase activity was low in samples (E,F and C) indicating improper storage, improper temperature, heat treatment, or adulteration with artificial sugar **(Afshari *et al.*, 2022).**

According to **Guler *et al.* (2014)** the lower enzyme content in honey is due to feeding bees with glucose, which likely enhances the deficiency of diastase that converts glucose and fructose.

Hydrogen Peroxide (H_2O_2) in honey:

The results in Table No (4) showed that the highest concentration of (H_2O_2) as follows as clover honey (D) from Tanta (152.90±0.12), citrus honey (A) from Buhaira Governorate (112.56±0.22), and citrus honey (B) from Kafr El-Zayat (96.73±0.04), respectively. The other samples are as follows : clover honey (E) from El-Mehala (67.84±0.02), citrus honey (C) from Qalyubia Governorate (39.23±0.58), and clover honey (F) from Upper Egypt (20.72±0.02).

The results showed that (H_2O_2) levels in honey samples changed significantly across samples of the same plant origin, and samples of different plant origin, depending on the dilution ratio. Use of buffered honey solutions can also raise hydrogen peroxide levels, and (GOX) activity is known to be (pH) dependent, with maximum activity at pH 6.1 **(Schepartz and Subers, 1964).**

Table (4): Activity of enzymes in honey (mean± SE).

Type	Place	(DN) Diastase	H_2O_2
Citrus	**(A) Buhaira Governorate**	**$11.36±0.03^b$**	**$112.56±0.22^b$**
	(B) Kafr El-Zayat	**$5.30±0.01^c$**	**$96.73±0.04^c$**
	(C) Qalyubia Governorate	**$2.81±0.02^f$**	**$39.23±0.58^e$**
Clover	**(D) Tanta**	**$14.06±0.04^a$**	**$152.90±0.12^a$**
	(E) El-Mehala	**$3.30±0.01^d$**	**$67.84±0.02^d$**
	(F) Upper Egypt	**$2.90±0.01^e$**	**$20.72±0.02^f$**

There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

(DN) Diastase Activity, (H_2O_2) Hydrogen Peroxide.

Conclusion and Recommendations:

The current study revealed differences in the six honey samples examined; thus, there were significant differences in the quality of honey samples of the same type, this is due to the difference in geographical location, the difference in plant origin, and the possibility of fraud by adding sugar to bees at the time of nectar harvesting, or by mixing a percentage of the sugary solution directly with honey, poor storage, and wrong practises by some beekeepers during honey harvesting, transportation and circulation.

We conclude from the above that there was a difference in the concentration of lead in honey samples according to the sites from which they were collected. Therefore, bee honey can be used as an indicator of lead pollution in the environment.

The recommendations were as follows:

1- The need to avoid placing apiaries near trafficked places crowded with cars.

2- The necessity of conducting a test to detect the sucrose content in honey, because, recently, methods of cheating honey by using sugar have spread, as well as manufactured honey, and also the addition of chemicals that decompose sucrose into glucose and fructose.

3- The surrounding environmental conditions, in terms of temperature and air humidity, must be taken into account while harvesting honey, as well as during storage, because honey absorbs moisture from the surrounding air.

4- The importance of keeping the containers in which honey is stored clean for long periods, such as containers made of tin and plastic, to avoid the high percentage of heavy metals in stored honey.

5- Beekeepers should be made aware of the dangers of heavy metal contamination, pesticides used in agriculture, and medicines used to treat various bee diseases.

6- The need to buy honey from reliable and approved sources.

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الخصائص الفيزيائية والكيميائية لعسل الموالح وعسل البرسيم التي تم جمعها من مناطق مختلفة بجمهورية مصر العربية

غادة فؤاد بدران[1]، سعاد توفيق خير[2]، محمد رضا بدر[1]، محمد بسيم عطا[1]

[1] قسم علوم وتكنولوجيا الأغذية ، كلية الزراعة ، جامعة طنطا ، مصر
[2] قسم علوم وتكنولوجيا الأغذية والألبان، كلية الزراعة ، جامعة دمنهور ، مصر

مجلة العلوم الزراعية والبيئية المستدامة

الملخص

يعتبر العسل منتج طبيعي، فهو يستخدم عادة لما يتميز به من حلاوة الطعم، كما أنه يعطي طاقة للجسم، وله فوائد صحية عديدة، وقد انتشرت في الآونة الأخيرة عمليات غش العسل على نطاق واسع، وذلك قد ينتج عنه فقد للكثير من القيمة الغذائية للعسل، كما أنه يشكل خطراً على الصحة العامة للمستهلكين، ومن الجدير بالذكر أن الخصائص الفيزيائية والكيميائية للعسل أيضاً تتأثر وفقاً لدرجة الغش الحادث فيه، لذلك هدفت هذه الدراسة إلى تقييم جودة العسل المصري في السوق المحلي، من خلال مقارنة نوعين من العسل (عسل الحمضيات وعسل البرسيم) ،وهذه العينات قد تم جمعها من المناحل، في مناطق مختلفة خلال مواسم 2021-2022.

وقد أظهرت النتائج أن جودة العسل، والتي تشمل الخواص الفيزيائية والكيميائية له، والنشاط الإنزيمي، ومحتوى المعادن الثقيلة في العينات، قد اختلفت باختلاف الأصول النباتية الجغرافية والبيئية. كما وجد أن درجة حموضة العسل أيضًا، قد اختلفت باختلاف الموسم الذي جمعت فيه العينات، حيث يتراوح الرقم الهيدروجيني لمعظم العينات من (3.40 إلى 6.10) وذلك طبقاً لحد الحموضة القياسي. واتضح أن عسل الموالح كان هو الأكثر حمضية، (فالرقم الهيدروجيني له = 3.33). وكان تركيز السكروز في عسل الموالح التجاري الذي تم جمعه من القليوبية يساوي (12.34٪)، وهذا يعتبر أعلى من تركيز عسل البرسيم الذي تم جمعه من صعيد مصر، وكان يساوي (9.75٪). ومن المثير للاهتمام أن نلاحظ أن بعض العينات التجارية كانت تحتوي على هيدروكسي ميثيل فورفورال، كما تحتوي على نسبة أقل من البرولين، وأيضاً أنشطة دياستيز أقل من الطبيعي، مما يشير إلى أن العسل كان يتم تسخينه أو تخزينه في ظروف سيئة أو أنه مغشوش.

الكلمات المفتاحية:
نحل العسل – الخصائص الفيزيائية والكيميائية – هيدروكسي ميثيل فورال - الغش