

Study of Physicochemical Properties of Some local and Imported Honey in Sebha

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Abstract: To compare the local honey product with imported honey in terms of nutritional importance, the research aimed to study some of the physical and chemical properties of 16 honey samples, 4 fresh samples from farms in southern Libya (Sabah City), and 12 samples imported from outside Libya, which are Al - Shifa honey from Saudi Arabia, Lune de miel from France and miel from Spain. The purpose of the study was to study some of the properties and chemical properties of these compounds. The results of the study showed that: The value of the refractive index is between (1.4908–1.4937), the percentage of moisture is between (17.07–18.20%), which is less than the allowable limit of international standards (<23%), European standards (<21%) and Libyan standards (<17%), the total solids are between (81.80-82.93%), and the soluble solids are between (80.93 -81.90), and the insoluble solid content was between (17.07-18.20%). Between (0.87-1.47%) this percentage was higher than the specified allowable limit international standards (<0.5%), European standard specifications (<1%) and Libyan standard specifications (<0.1%), the proportion of total ash was 0.14-0.32 %, but the proportion of total ash and its contents was the highest in fresh honey samples and samples imported from Spain. It was the lowest in samples imported from France (lune de miel), with a percentage of total acidity between 0.29 and 0.39%, except for ash dissolved in water.

The total sugar content was between (56.73-74.54%) and the reduced sugar content was between (41.56-69.02%), below the acceptable limits of Libyan standards, except for the French sample (lune de miel), which was within the acceptable limits. The proportion of non-reducing sugars, glucose and fructose is as follows: (5.24-18.85%, 13.67-42.25%, 2.81-42.28%), Each. Experiments to detect the presence of cheating in honey did not show the effect of cheating in the samples studied.

Keywords: Cheating, Fresh, Honey, Humidity, Imported, Physicochemical.

Introduction

Honey bee Characters

Bee honey is a sweet, thick liquid that bees use as a natural food. It can be said that honey is the nectar and sugary substances that worker bees collect from plants, and they modify and store them so that the moisture content in them does not exceed 25%, the ash percentage is less than 0.25%, and the sucrose percentage is less than 08%. This definition is considered the most accurate [1, 2]. Honey is classified according to bee food into several types such as hawthorn honey, chestnut honey, eucalyptus honey, orange honey, rosemary honey, linden honey, clover honey and others [3].

The natural characters of bee honey and its chemical composition

It is very rare to get two samples of honey that are completely similar in all the properties and characteristics, and the main factor causing the difference is the floral source in addition to other factors such as soil type, weather factors and methods of preparing honey.

The natural characters of honey

The density of honey means the weight of honey in relation to the volume unit, which is on average 1.1-1.2 g/mL. As for its colors, it has been placed in two groups, the first for the white color with its degrees and the second for the amber color with its degrees [1, 2]. The crystallization periods of honey vary in different types, some crystallize During production, while some remain in liquid form for several years, the rate of crystallization is affected by the ratio of dextrose to levulose. When its molecules acquire water, it turns into dextrose hydrate, which contains 9% water, and thus the percentage of dextrose decreases, and the crystals separate from the honey and precipitate, and its crystallization is affected by colloids, moisture, and storage temperature [4,5].

As for the viscosity of honey, it means the speed of its flow, and it is affected by several factors, the most important of which is the water content. The greater the amount of water, the lower the viscosity, which is inversely proportional to the temperature, up to 35°C, where it decreases significantly, and its decrease becomes unimportant if it rises above 45°C [1]. The viscosity of mature honey is higher than that of unripe

honey, as ripe honey contains 14-18% water, which should not exceed 20% [5].

Fermentation of honey occurs as a result of the presence of some types of yeasts that live in high concentrations of sugars. Fermented honey can be distinguished by its pungent taste and the appearance of white spots on its surface.

Fermentation occurs when honey is immature and its moisture is higher than usual. When its percentage is less than 17% it does not encourage fermentation, but if it increases it leads to the possibility of its occurrence, and if it exceeds 19% it must occur and the optimum degree of activity of yeasts 11°C or slightly higher [4]. If honey is stored in a dry place at a temperature not exceeding 10°C the air humidity is usually low and honey does not ferment [6].

Carbon dioxide is the product of honey sugar fermentation and appears in the form of foam or large air bubbles, and alcohol turns into acetic acid and water if honey is contaminated with bacteria in the presence of oxygen, and thus the taste of honey becomes acidic [7].

Chemical composition of honey

Honey varies according to the plant source from which it is collected, in addition to other factors. It was found that the nectar of flowers contains different percentages of several sugars, the percentage in nectar varies between 22-40% [4].

honey composition

The water content where the amount of water remaining in the nectar after its full maturity and its transformation into honey is known with the natural honey moisture and ranges between 13.4-22.9% with an average of 17.20% and the water content may change after sorting operations depending on the storage conditions. Its proportion affects the natural properties of honey [1]. It also contains 76% of sugars, 74% of which are glucose and fructose [6]. The high concentration is credited with inactivating different types of bacteria and many other fungi [1]. It is considered a source of energy as the sugars in honey have a sweetness that exceeds the sweetness of cane sugar by 25%. Also, 3.79 liters of filtered honey contains an average of 1.14 kg of sugars, which is equivalent to the sweetness of 1.40 kg of cane sugar, and each tablespoon of honey contains about 60 calories [7].

Honey also contains a very small percentage of ash estimated at about 0.17% on average with a range between 0.02-1.028% and a large number of high-value elements, such as K, Ca, Na, P, Mg, Fe, Co, Mo, V and Ag that plays an important role in metabolic and genetic processes [6]. It also has an alkaline effect that lowers stomach acidity [1]. It was found from the

spectroscopic analysis of honey that it contains salts and elements Mn, Si, Al, Br, Cr, Cu, Ni, Pb, Sn, and some types of it contain Ra and the percentage of mineral salts in honey is almost equal to that in human blood serum [5].

There are many organic acids and proteins in honey and they differ depending on its source, including citric, formic, acetic, tannic, oxalic and gluconic acid which the most important [8, 9]. Acids make up a very small percentage of honey, about 0.57% and greatly affect its taste [1]. It also contains a small percentage of proteins that include albumins. Its presence is considered one of the advantages of natural honey [7]. It was found that one kilogram of honey contains several vitamins such as vitamin B₁, B₂, B₃ and C, with an amount of 0.1, 1.5, 2 and 30-54 mg, respectively [8], and the hydrogen ion pH of honey ranges between 3.29-4.87% [7].

In honey there are very small amounts of substances that are secondary components such as some dyes, in addition to flavor and aroma additives and enzymes [4] where honey contains several enzymes such as diastase, catalase and invertase in addition to some other enzymes that accelerate the decomposition of complex foods into simple ones that are easily absorbed [6]. What distinguishes honey from other sugars is the presence of these enzymes that bees are a source of, and it may be due to pollen and nectar, or perhaps to the presence of some types of yeasts that tolerate high concentrations of sugars or some types of microorganisms. There are enzymes that are likely to be present in honey, such as inulase, catalase, and phosphatase [1], and when honey is heated at a high temperature for a long time the enzymes are damaged and this is what happens during the manufacture and storage of honey for a long time [7].

Advantages of honey

Compared to the types of sugars used in food, honey is characterized as not causing disturbances to the thin digestive tract membranes, and is represented in the body quickly and easily. It has a natural effect, as it facilitates the process of excretion, does not harm the kidneys or damage its tissues and provides the body with activity units with less trauma to the digestive system, and it also helps athletes to renew their activity [10].

Benefits and therapeutic properties of bee honey

In addition to being a source of energy, it is rich in fructose, a sugar whose digestion does not require any intervention by insulin. It also addresses weakness and early weakness of vital body functions, activates burns and wounds healing, and reduces pharyngeal sensitivity [3, 11]. It can be used to prevent and treat anemia, and although honey is slightly acidic, it works

to find alkalinity in the blood. It was tested that many types of bacteria perish as soon as they come into contact with honey [10]. It is also considered an anti-inflammatory, anti-bacterial, anti-microbial and biological, as it is used in the treatment of gum fungi, mouth odor, bed sores, eye wetness, interdigital fungi, sinusitis and psoriasis, as well as the treatment of leishmaniasis. The effectiveness of honey and some herbs has also been tested for the treatment of some cases of hepatitis (C and B Hepatitis) and succeeded in treating it and eradicating it permanently in some cases, he also succeeded in reducing the percentage of virus in the blood when the analysis was done by PCR [12].

Materials and methods of analysis

Solutions

Standard solutions of N0.1 and 40% sodium hydroxide, 0.1N hydrochloric acid, concentrated sulfuric acid, 0.1 N sodium thiosulfate, saturated solution of neutral lead acetate, copper sulfate, potassium iodide 15%, methylene blue indicator 1%, starch indicator 1%, methyl orange indicator, phenolphthalein 0.5% indicator were prepared as mentioned [13]. Buffer solutions, Fehling's solution A and B, and sodium oxalate, the salts and solutions used were of high purity and were produced by Merick Company.

glassware and hardware

Glassware needed to work in addition to bottles and filter papers.

Sensitive balance made in China, electric heaters, burning furnace, water bath, pH Jenway Ion Meter Model 3205, Refractometer (60/70 ABBE) both British made. The analyzes were carried out in the laboratories of the Department of Chemistry, Faculty of Science, Sebha University, Libya

Sample collection

Honey samples were obtained (randomly) from the local markets in the city of Sebha, and their number was 16 samples (L), a Libyan sample obtained from a farm inside the city of Sebha, sample (S) Al-Shifa honey imported from Saudi Arabia, sample (F) (Lune de miel) Imported from France and sample (A) (Miel) imported from Spain.

Analysis methods

Humidity

It was estimated in honey samples using a measure of the optical refractive index, which was estimated by a laboratory Refractometer, according to what was mentioned in [14], which depends on the change in the properties of rays when they pass through two different media in optical density. The moisture

percentage is obtained after correcting the temperature Heat to 20°C [15,16].

Total solids

It was estimated by subtracting the percentage of moisture from 100.

dissolved solids

It was estimated using a Refractometer and the method previously mentioned for estimating humidity.

insoluble solids

Calculated by subtracting the percentage of solutes from the percentage of total solids.

Total acidity

It was estimated by titrating a weight of honey dissolved in a volume of CO₂ free distilled water by NaOH (0.1N) solution and phenolphthalein and the result was calculated based on the acid predominating in honey [14].

pH

It was estimated for a prepared solution of honey in carbon dioxide-free distilled water using a pH meter set using two buffer solutions pH 4 and 7 [17].

total ash

It was estimated by taking a weight of honey and burning it at 425 °C in a crucible until it turned into white ash and the weight was fixed.

Water insoluble ash

It was estimated as mentioned in [14] by adding 25 mL of deionized water to the white ash in the crucible and heating it for 5 min. Then it was left to cool and filtered on an ashless filter paper. The filter paper and the precipitate were placed in a crucible and introduced to the incineration furnace. After the burning process was completed, the crucible was transferred to a dryer and weighed after it had cooled down, and the process was repeated until the weight was stable.

Ash dissolved in water

% for ash soluble in water = % for total ash - % for ash not soluble in water.

Alkaline ash

drops of orange methyl indicator were added to the filtrate and titrated with 0.1N HCl.

Reducing, total and non-reducing sugars

2 g of honey was weighed and quantitatively transferred to a 250 mL standard flask using 100 mL of distilled water, then the leaching process was carried out using a saturated solution of neutral lead acetate in a 250mL standard flask, then sodium oxalate was added to get rid of the excess of lead. (Lane as stated in [15].

Reducing sugars

5 mL of Fehling's solution A and 5 mL of Fehling's solution B were transferred to an 250mL Erlenmeyer

flask and immediately after boiling 15mL of the sugar extract in the burette was added and the extract was dipped until all the copper was completely reduced, (using 1% methylene blue indicator as the redox).

Total sugars

50 mL of clear sugar extract was taken, transferred to a standard flask with a capacity of 100 mL, 10 mL of distilled water and 10 mL of dilute HCl (1:1) were added to it and placed in a water bath at 65°C for 10 min. Then it was neutralized by 40% NaOH with the use of Phenolphthalein indicator, the beaker left to cool and then completed with distilled water to the mark and the sugars were estimated in the same way as before.

Non-reducing sugars

The non-reducing sugars were estimated as sucrose as follows:

% for non-reducing sugars = % for total sugars - % for reducing sugars \times 0.95.

Glucose and fructose were measured using the Shaffer Hartman method.

5 gm of honey sample was taken and transferred quantitatively to a standard 250 mL flask and completed with distilled water to the mark, then 2 mL was taken from the dilute solution and transferred to a Erlenmeyer flask, 5 mL of Fehling's solution A and 5mL of Fehling's solution B were added and diluted to 10 mL with distilled water, and heated on a water bath and boiled for 15 min. After cooling the contents of the flask, 20 mL of concentrated 2N sulfuric acid was added, then 20 mL of a 15% potassium iodide solution was added. After vigorously shaking the beaker, the free iodine is isolated and titrated with $\text{Na}_2\text{S}_2\text{O}_3$ (0.1N) using 1% starch as a indicator, and the number of sodium thiosulfate used and the number of excess copper equivalents from the reaction.

A standard experiment was conducted (using distilled water instead of Blank honey solution), from which the number of milligrams of copper that was reduced by sugar was calculated, and from the tables of (Hartman Shaffer) the number of milligrams of glucose corresponding to the number of milligrams of copper was obtained, from which the percentage of glucose in honey was calculated. By knowing the percentage of reducing sugars and the percentage of glucose, the percentage of fructose in honey can be calculated [15].

% for fructose = % for reducing sugars - % for glucose

Honey cheat

Honey is cheated by adding water, starch, sugar solutions or molasses (as there are industrial honeys). In nineteen samples of industrial honeys, it was found that the percentage of Hydroxyl methyl furfural

ranges between (0.008-0.14%), while there is no percentage of it in the natural honeys [4].

Methods for detecting fraud in honey

Honey is cheated with commercial glucose or cane sugar, where cane sugar is analyzed in an acidic medium to give a sugary solution whose components are largely identical to natural honey even in the proportions of its presence, and it is difficult to detect fraud in this case, which led to the development of detection methods that measure the extent to which a commercial sugar solution is added. Since natural honey is equal to the rotation of the polarized light (Levorotatory), i.e. counterclockwise, it is possible to use a simple method to detect adulterated honey, which is Polariscopes), as the addition of cane sugar or commercial glucose changes the direction of rotation from left to right and becomes Dextrorotatory honey clockwise.

The Buckman test is one of the best methods to detect cheating by adding commercial glucose, where honey is diluted by equal weight with distilled water and then treated with potassium iodide (KI) solution. If it turns red or purple, this indicates the presence of adulteration in commercial glucose [18].

Cheating by adding water estimated by the percentage of moisture, if it exceeds 20%, it is considered adulterated [7], or by using ultraviolet rays. Honey adulterated with water absorbs less of it, unlike natural honey which absorbs a larger amount and cane sugar absorbs less than glucose and fructose absorbs [10]. As for cheating by adding starch, it is indicated by the addition of iodine, and the blue color is evidence of cheating.

Simple and quick ways to detect fraud in honey is the method of burning is by placing a drop of honey on a newspaper paper and burning it. If the drop burns and becomes a dark brown color instead, this indicates the presence of table sugar in honey. If the fire turns around the drop, this means that it is not cheated.

The uninterrupted thread method is done by dipping the finger in honey and raising it. If it flows in the form of intermittent threads, it indicates the presence of cheating, and if it flows in the form of a continuous thread, it indicates its purity [19].

Statistical analysis

The experiments were designed with four replicates for each type, and the statistical analysis program (ANOVA) and (SPSS program) were applied to the results, and they were treated as data in the entire randomized sector, and the least significant difference (L.S.D.) was calculated at the level of significance of 0.05 According to what was mentioned by [20].

Results and discussion

The estimation of the physical and chemical composition of the samples including refractive index, moisture, solids, ash, sugars, pH, total acidity and fraud detection were included in tables (1 and 2).

Table 1: Physical and chemical analysis of honey samples (gm/100g) based on wet weight.

Honey type Component	L	S	F	A	L.S.D.
Refractive index	1.4932 ^b	1.4912 ^c	1.4937 ^a	1.4908 ^d	0.004
Humidity	17.20 ^b	18.00 ^a	17.07 ^b	^a 18.20	0.220
Total solids	82.80 ^a	82.00 ^b	82.93 ^a	81.80 ^b	0.220
Dissolved solids	81.33 ^{ab}	80.97 ^b	81.90 ^a	80.93 ^b	0.630
Insoluble solids	1.47 ^a	1.03 ^a	1.03 ^a	0.87 ^a	N.S.
Total ash	0.32 ^a	0.24 ^b	0.17 ^c	0.14 ^c	0.050
Water insoluble ash	0.16 ^a	0.14 ^{ab}	0.15 ^a	0.10 ^b	0.050
Ash dissolved in water	0.16 ^a	0.10 ^b	0.02 ^c	0.04 ^c	0.050
Alkaline ash dissolved in water	3.98 ^a	2.49 ^b	2.11 ^c	1.95 ^c	0.030
Total acidity	0.29 ^b	0.39 ^a	0.39 ^a	0.32 ^{ab}	0.076
pH	4.52 ^a	3.43 ^c	3.72 ^b	3.74 ^b	0.130

(N.S.) There is no difference between samples with the same symbol (d-a) at the level of significance ($P \leq 0.05$)

Refractive index

From Table (1) the value of the refractive index ranged between 1.4908-1.4937, and it was the highest for the French honey sample (F) and the lowest for the Spanish honey sample (A). (L), then (S), and finally (A), and the results obtained were within the range obtained in the study [21].

Humidity

The percentage ranged between 17.07-18.20% and was the highest for sample (A) and lowest for sample (F). The statistical analysis showed that there were no significant differences between the two samples (S) and (A) as well as between the two samples (F) and (L), but it showed that there are significant difference between the two samples (S) (A) and samples (L) and (F), the obtained values were less than the limit stipulated in the International Standard (>23%) and the European Standard (>21%) [22] but higher than the limit stipulated in the Libyan specification (<17%).

Total solids

The percentage ranged between 81.80-82.93%, and the sample (F) recorded the highest value and the sample (A) the lowest value. The statistical analysis did not show significant differences between the two samples (L) and (F) and the two samples (S) and (A), but it showed a significant difference between samples (L) and (F) compared with samples (S) and (A).

Dissolved solids

The percentage ranged between 80.93-81.90% and the upper value of the sample (F) and the lower value of

the sample (A), the statistical analysis did not show any significant differences between the sample (L) compared to the samples (S), (F) and (A), but statistically the significant difference appeared between sample (F), sample (S), and sample (A).

Insoluble solids

The percentage ranged between 0.87-1.47% and the highest value for the sample (L) and the lowest value for the sample (A), the statistical analysis showed that there were no significant differences between all samples and the obtained values were higher than the permissible limit in the international standard not exceeding (0.5 %), the European Standard does not exceed (1%) and the Libyan Standard does not exceed (0.1%).

Total ash

The percentage ranged between 0.14-0.32%, it was the least significant for sample (A) and the highest significant for sample (L), the statistical analysis did not show a significant difference between the two samples (A) and (F), which recorded the values 0.14 and 0.17%, respectively, the significant difference appeared between samples (S) and (L), as well as between samples (A) and (F), the percentage of total ash was lower than that obtained in the study [21].

Water insoluble ash

The lowest percentage was 0.10% and the highest was 0.16% for samples (A) and (L), respectively. Statistical analysis did not show significant differences between samples (F), (L) and (S), as well as no significant difference between samples (A) and (S). Significant difference appeared between samples (F), (L) versus sample (A).

Ash dissolved in water

From Table 1, we note that 0.02% was the lowest percentage recorded by the sample (F), while 0.16% was the highest percentage recorded by the sample (L). The statistical analysis showed that there were no significant differences between samples (F) and (A). The statistical analysis showed that there were significant differences between Samples (S) and (L) and between samples (F) and (A).

Alkaline ash dissolved in water

It ranged between 1.95-3.98 mLEq./gm100, where it was the lowest significant for the sample (A) and the highest significant for the sample (L). Statistical analysis showed that there were significant differences between the two samples (L) and (S) and between them and the two samples (F) and (A), while there was no significant difference between the two samples (A) and (F).

Total acidity

Table 2: Results of analysis for sugars (g/100g) based on wet weight

Honey type Component	L	S	F	A	L.S.D.
Total sugars	61.41 ^c	56.73 ^d	74.54 ^a	65.96 ^b	4.32
Reducing sugars	55.95 ^b	48.49 ^c	69.02 ^a	41.56 ^d	4.05
Non-reducing sugars	9.52 ^b	7.82 ^{bc}	5.24 ^c	18.85 ^a	3.26
Glucose	13.67 ^b	42.25 ^a	41.58 ^a	38.75 ^a	5.83
Fructose	42.28 ^a	6.24 ^c	27.44 ^b	2.81 ^c	5.12

(N.S.) There is no difference between samples with the same symbol (d-a) at the level of significance ($P \leq 0.05$)

Total sugars

The percentage ranged between 56.73-74.54%, the lowest value was for the sample (S) and the highest for the sample (F), the statistical analysis showed that there were significant differences between all samples.

Reducing sugars

The percentage ranged between 41.56-69.02%, the lowest value was for sample (A) and the highest value was for sample (F), statistical analysis showed no significant differences between samples (A), (S), (L) and (F). The percentages were less than the permissible limit in the Libyan standard, except for the sample (F), which was within the permissible limit.

Non-reducing sugars

The percentage ranged between 5.24-18.85%, the lowest was for the sample (F) and the highest for the sample (A). The statistical analysis showed that there was no significant difference between the two samples (S) and (L) as well as no significant

The percentage ranged between 0.29-0.39%, the highest value was for the sample (F) and (S) and the lowest value was for the sample (L), the statistical analysis did not show significant differences between the two samples (F) and (S) compared to the sample (A) as well. Significant difference between the sample (L) and the sample (A), while the significant difference only appeared between the eyes (F) and (S) compared to the sample (L). The obtained values were low compared to the values of the study [21].

pH

The pH degree ranged between 3.43-4.52 for the two samples (S), (L) respectively, the values were 3.43, 3.72, 3.74 and 4.52 for the samples (S), (F), (A) and (L) respectively, the statistical analysis did not show a significant difference between the two samples (F) and (A), but the significant difference appeared between them and the sample (L) and the sample (S).

difference between the two samples (F) and (S) and the significance differences appeared between samples (A), (L) and (F).

Percentage of glucose

The percentage ranged between 13.67-42.25%, it was the least significant for the sample (L) and the highest for the sample (S), the statistical analysis did not show any significant differences between samples (A), (F) and (S), but the significant difference appeared between them and the sample (L).) with a value of 13.67.

The percentage of fructose

The percentage ranged between 2.81-42.28%, it was the least significant for sample (A) and the highest significant for sample (L), the statistical analysis did not show any statistical difference between the two samples (A) and (S), but the significant differences were recorded between the two samples (F) and (L) versus samples (A) and (S). The obtained values were less than the upper limit mentioned in the study [21].

Cheat detection

The analyzes proved that there was no cheat of any kind mentioned in the samples.

Results

The analyzes showed that the percentage of moisture in the Saudi and Spanish samples is higher than the permissible limit in the Libyan standard, and this makes it susceptible to fermentation.

The insoluble solids are higher than the permissible limit in the Libyan, European and international standards.

The reducing sugars for the French sample only were within the limit allowed in the Libyan standard.

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