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# ANALYZING THE PHYSICOCHEMICAL PROPERTIES OF HONEY BEES FROM VARIOUS SOURCES AND QUANTIFYING GLUCOSE AND FRUCTOSE USING HPLC

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## Abstract

Honey is a sweet, sticky substance that is produced by bees after nectar and honeydew have been collected. This study was aimed to determine the physiochemical properties of native and imported honey in Iraq to evaluate the quality of various types of honey. Physio-chemical investigations of these samples were done utilizing AOAC protocols and assessed according to Codex Alimentarius Commission and European Union Council instructions. The moisture contents ranged from 15.16 ± 0.15 to 19.92 ± 0.67 %. Ash concentration of all samples was ranged from 0.42 ± 0.01 to 1.61 ± 0.09 %. The levels of pH in all tested honey samples were acidic and within the standard range. Saudi honey showed the highest free acidity values (49.51 + 0.18 meq/kg), whereas the lowest free acidity value was recorded in Turkish honey (21.71 ± 0.11 meq/kg). Electrical conductivity (EC) in the analyzed honeys ranged from 0.57 ± 0.07 to 1.26 ± 0.01 mS/cm. In both glucose and fructose, the highest average levels were recorded in the Iraqi honey sample where they reached (30.25 ± 0.05 and 40.18 ± 0.13 %) respectively, while the lowest content of both glucose and fructose were recorded in the Saudi honey samples (26.88 ± 0.07 and 36.23 ± 0.07%) respectively. For all of the samples studied, the estimated fructose to glucose percentage ranged from 0.51 ± 0.06 to 1.69 ± 0.09. Iraqi, Turkish and Saudi honey samples have greater HMF levels than Iranian boney, and the three samples were within the standard limit (not more than 40 mg/kg). Honey quality differed depending on geographical and floral origins, seasons, transportation, processing conditions, and storage time. The glucose-fructose ratio was determined using high-performance liquid chromatography.

**Keywords**: *Honey, Physicochemical properties, HPLC.*

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## Introduction

Honey (pronounced /hni/ in English) is derived from the Germanic word "hunaga," which means "golden". It's described as a "naturally sweet and viscous fluid obtained mostly from flower nectar or excretions on living plant parts(Sobhy A El Sohaimy et al., 2015). Honeybees (Apis mellifera) collect these substances by regurgitation and evaporation, which are then stored and left to ripen and mature in the honeycomb (Commission, 2001). Honey quality is mainly evaluated by its physicochemical besides microbial properties, and the physicochemical characteristics of honey are specified in national and international standards. Honey quality, composition, and biochemical qualities are influenced by ripeness, harvest season, climate conditions, production and processing processes, storage period and location, and source of nectar (single or multiple flowers) have significant impact on the quality, components and biochemical feathers of honey. These parameters can have different physicochemical properties. The essential properties of honey are moisture, electrical conductivity, ash, pH acidity diastatic activity, hydroxymethylmorphoreal and proline content(Sobhy A El Sohaimy et al., 2015). In light of the foregoing, there are both unbranded and branded honeys available in the market. There could be large variances in honey brands in terms of nutritious value in addition to honey quality. The majority of consumers are unaware of the kind of honey they are consuming, because of the rising concerns associated with adulteration and tampering with natural honeys supplied on the market, it is critical to conduct a quality examination of commercial honey. Hence, this research was aimed to assess physicochemical properties of local and imported honey in Iraq to evaluate the different types of honey quality. The HPLC's separation technique has been on the basis of equilibrium that is created between molecules existing in mobile phase, and they have been maintained in stationary phase(Silva et al., 2019).

**Table 1**: International Standard of Codex Alimentarius.

| NO | Parameter | Normal Range |
|---|---|---|
| 1 | Moisture | 13.4-19.0% |
| 2 | pH | 3.42-8.10 |
| 3 | Free acidity meq/kg | Not less than 45 |
| 4 | Total Fructose and Glucose % | Not rore than 75 % |
| 5 | Electrical Conductivity mS/cm | 0.8-1.6 Ms/c |
| 6 | High hydroxy methyl furfural (HMF) mg/kg | Not more than 80 mg/kg |

## 2. Materials and methods

### 2.1. Collection of samples

In this study, four different multi-flower honey brands, the most commonly consumed ones in Iraq, collected from market places in January 2021. Three of these brands were representing Turkish, Iranian and Saudi honey (with close production dates ranging between December 2020 and June 2021), while one honey sample was from the mountainous region in Sulaymaniyah governorate represented the Iraqi honey sample. All of the honey samples were kept at room (22-24 °C), until analyses (G. Hegazi et al., 2018).

### 2.2. Physicochemical investigation of honey

Selected physicochemical parameters in current study, including moisture percentages, ash concentration, pH and free acidity, glucose and fructose levels, electrical conductivity ratio as well as hydroxymethylfurfural (HMF) content are performed by Materials Research Unit/Ministry of Science and Technology in Baghdad.

#### 2.2.1. Determination of moisture concentration

According to(Al et al., 2009), Moisture value of all experimental honey samples was assessed by refractometry, all examinations carried out at 20 °C.

#### 2.2.2. Determination of ash content

Approximately 5 g of each honey sample was placed in combustion pots, and the samples were burnt in a burning muffle for 5 hours at a high temperature (550 °C), the process was repeated until the weight was stable (Produced et al., 2016).

#### 2.2.3. Determination of pH and acidity

The pH and acidity of each honey sample were determined using the following methods: In a 250 mL beaker, Weight 10 g of each honey specimen in 75 mL distilled water. The pH determined via pH meter, and with the 0.1 N sodium hydroxide (NaOH) solution, the honey suspension was titrated till pH 8.3 (Al-farsi et al., 2018).

Acidity % = Weight of Honey x Vol of NaOH

#### 2.2.4. Determination of electrical conductivity (EC)

The electrical conductivity (EC) was determined utilizing an electrical conductivity meter (EC meter) in the following way: 10 g of each sample was weighed perfectly and dissolved in 75 mL distilled water and shaked for 30 minutes at 37 °C. Reading is recorded after the stabilization of the device (Sistematik et al., 2021).

#### 2.2.5. Determination of glucose and fructose

Qualitative and quantitative of fructose and glucose is achieved according to association of the official analytical chemists (Produced et al., 2016). One gram of each specimen are diluted in few milliliters of purified water, filtered through membrane 0.45 µm filter and directly injected in the high performance liquid chromatography (HPLC) an

equipment with UV detector. The separation is carried out utilizing carbohydrate analysis column (3.9×350 mm: 10 µm particle size) with mobile phase 80% acetonitrile ($CH_3CN$) in 20% water, flow rate 2 ml/min; injection volume 25 µl as shown in table 2.

**Table 2:** The preliminary chromatographic conditions of the methods suggested.

| **Preliminary chromatographic conditions** | |
|---|---|
| **UV-Detector** | 254 nm |
| **Injection volume** | 25 µL |
| **Flow rate** | 2 mL/min |
| **Temperature** | 25 °C |
| **Mobile phase** | ACN 80% /Water 20 % |

### 2.2.6. Determination of hydroxyl-methylfurfuraldehyde (HMF)

Five grams of each sample are diluted in distilled water up to 50 ml, passed through a 0.45 µm filter, and injected into a HPLC device. The separation is carried out utilizing analytical column (125x4 mm) with a particle size diameter of 5 µm. The HPLC circumstances: mobile phase (80% water) and (20% acetonitrile $CH_3CN$); the injection volume of the sample was 20 µm. with a flow rate of 0.7 ml/min (Enedettia et al., 2004).

### 2.2.7. Statistics

The statistical analyses were done in triplicates, least significant difference LSD test (Analysis of Variation-ANOVA) was utilized to compare the quantified variables between the honey samples. All analyses were carried out using the SPSS Statistic software.

## 3. Results and discussion

The results of physicochemical analysis of all the examined honey samples local and imported are given in the Tables 2.

**Table 2**: Physicochemical properties (Mean ± SD) of four honey samples (n = 3).

| O | Physicochemical properties | Honey type | | | | LSD value |
|---|---|---|---|---|---|---|
| | | Iraqi | Turkish | Saudi | Iranian | |
| 1 | Moisture % | 15.16 ± 0.15 a | 15.68 ± 0.03 c | 21.94 ± 0.17 a | 19.92 ± 03 b | 0.1266** |
| 2 | Ash % | 1.12 ± 0.08 a | 1.61 ± 0.09 a | 0.86 ± 0.05 b | 0.42 ± 0.01 b | 1.188** |
| 3 | pH | 3.49 ± 0.12 a | 3.71 ± 0.06 a | 3.88 ± 0.28 a | 3.16 ± 0.08 a | 0.206 NS |
| 4 | Free acidity meq/kg | 37.92 ± 0.35 b | 21.71 ± 0.11 c | 49.51 ± 0.18 a | 33.00 ± 0.06 b | 1.2247** |
| 5 | EC mS/cm | 0.57 ± 0.07 c | 0.89 ± 0.07 b | 0.83 ± 0.03 b | 1.26 ± 0.01a | 0.0039** |

| | | | | | | |
|---|---|---|---|---|---|---|
| 6 | Glucose % | 30.25 ± 0.05 a | 28.89 ± 0.03 b | 26.88 ± 0.07c | 29.91 ± 0.07b | 2.596** |
| 7 | Fructose % | 40.18 ± 0.13 a | 39.13 ± 0.10 a | 36.23 ± 0.07 c | 38.77 ± 0.08 b | 2.218** |
| 8 | Σ (Fructose and Glucose) (%) | 70.43 ± 15 a | 68.02 ± 11 b | 63.11 c | 68.68 ± 12 b | 2.699** |
| 9 | Fructose/Glucose (F/G) ratio | 1.55 ± 0.08 a | 1.69 ± 0.09 a | 0.51 ± 0.06 b | 1.36 ± 0.07 a | 1.181* |
| 10 | HMF mg/kg | 22.62 ± 0.02 c | 23.91 ± 0.26 c | 30.22 ± 0.18 b | 43 12 ± 0.15 a | 6.130 ** |

### 3.1. Moisture concentration

Percent moisture contents of the examined samples were 21.94 ± 0.17 % for Saudi, 19.92 ± 0.3 % for Iranian, 15.68 ± 0.03 % for Turkish and 15.16 ± 0.16 % for Iraqi honey samples respectively (Table 2). There were significant differences (P > 0.01) between examined samples. In general, the moisture level for Iraqi sample registered the lowest moisture value (15.16 ± 0.15 %) among tested samples, whereas the Saudi honey sample exhibited the highest moisture value (21.94 ± 0.17 %). The moisture level in honey is the quality criterion for its long-term stability and spoilage resistance to fermentation. Honey fermentation is more likely to occur at higher moisture levels during the period of storage. Codex Alimentarius has set an allowable moisture limit of 20% (Commission, 2001). Hence, the low moisture content, extends the shelf life of honey.

### 3.2. Ash content

The content of ash is a quality indicator for the phytogeographical origin of honey. Honey has low ash level and it differs depending on the substance gathered by the bees throughout foraging on the flowers. In the current work, Iranian and Saudi honey samples recorded the lowest values of ash content (0.42 ± 0.01 and 0.86 ± 0.05 %) respectively. Conversely, the highest levels of ash content were shown in Iraqi and Turkish samples. (1.12 ± 0.08 and 1.61 ± 0.09 %) respectively with remarked significant difference (P > 0.01) between examined samples. All samples had acceptable limits of ash concentration (0.6 - 1.2(Commission, 2001). The present findings showed approximately similarity with the results of (Isolates, 2015).

### 3.3.pH

In this study, the values of pH of all examined samples were acidic and within the acceptable limit (pH 3.40 - 6.10) (Commission, 2001). Despite the statistical analysis show non-significant differences between all studied honey samples, however the Saudi honey sample was the most acidic (pH 3.88 ± 0.28), Iranian honey, on the other hand, had the lowest acidity (3.16 ± 0.08) (Table 2).The results obtained were similar compared to data previously reported in Algeria and Oman honeys, which were between 3.49 and 4.70 (Draiaia et al., 2015)The acidity of honey is owing to the presence of organic acids such as

citric, tartaric, oxalic, acetic, and others. Moreover, it's also possible that the honey samples have a high mineral content(S A El Sohaimy et al., 2015).

### 3.4. Free Acidity

The results of this work showed a significant difference ($P < 0.01$) in the free acidity values between all examined honeys samples especially between Saudi and Turkish samples (Table 2). In general, the average of free acidity values for Turkish honey showed the lowest free acidity values (21.71 ± 0.11 meq/kg) among tested samples, followed by Iranian (33.00 ± 0.06 meq/kg) and Iraqi (37.92 ± 19.35 meq/kg), while the Saudi honey recorded the highest free acidity values (49.51 ± 0.18 meq/kg). The acidity of honey must not reach 40 meq/kg (Commission, 2001). Therefore, except for Saudi sample, which was not approved by the codex limits, all honey samples were within the standard range. The acidity value reflects the equilibrium between organic acids in honey, which varies depending on floral composition and bee species (Nayik & Nanda, 2015). The results obtained in the present study consented with data in the previous literature (Article, 2022).

### 3.5. Electrical conductivity EC values

The values for electrical conductivity (EC) of all analyzed honey samples exhibited that highest EC recorded in Iranian honey sample (1.26 ± 0.01 mS/ cm) (Table 2) which was slightly higher than standard range (that not more than 0.80 mS/cm) in accordance with the international standard stated by Codex Alimentarius Commission (Commission, 2001). In contrast, the EC levels in Iraq, Saudi Arabia, and Turkey honeys were the lowest (0.57 ± 0.07, 0.89 +0.04 and 0.83 ± 0.03 mS/cm) respectively with significant difference ($P < 0.01$) in comparison with the highest value of EC obtained Iranian sample. EC is one of most important parameters in determining of the physical properties of honey. This parameter is influenced by the concentration of ash, proteins, complex sugars organic acids, besides differs depending on floral origin. EC of the honey is directly associated to the total content of organic acids, mineral salts, and proteins; it's a variable that varies a lot depending on the botanical origin, and it's one of the greatest ways to express the difference between different kinds of (Albu et al., 2021).

### 3.6. Glucose & fructose

The relative quantity of fructose and glucose is critical for the judgment of honey quality (Buba et al., 2013). The data shown in Table 2 indicate that there were significant differences ($P \leq 0.01$) in the average of both glucose and fructose levels between the examined honey samples. In both glucose and fructose, the highest average level was recorded in the Iraq honey samples where they reached (30.25 ± 0.05 and 40.18 +0.13 %) respectively. Whereas the lowest average content recorded in the Saudi honey samples (26.88 ± 0.07 and 36.23 +0.07 %) respectively. Indeed, fructose was the main sugar in all honey samples, which could be due to partial oxidation of glucose to gluconic acid and hydrogen peroxide by the enzyme glucose oxidase(Zamanian & Azizi-Soleiman, 2020). These findings corroborated the findings of several earlier investigations on various honey

varieties(Salehi et al., 2019).

### 3.7. (Fructose and Glucose) (%)

The statistical investigation revealed a significant difference ($P < 0.01$) in the average total value on fructose + glucose concerning all the examined samples. Among all honey types, Iraq honey recorded the highest percentage (70.43 ± 15 %) followed by Iranian honey (68.68 ± 12%) and Turkish honey (68.02 ± 11 %), while the lowest total content was detected in Saudi honey (63.11 ± 0.03 %). The previously obtained results as shown in Table 2 explained that fructose and glucose are the main sugars in honey samples, which although restrictions have been set for their individual values, their sum (fructose and glucose) has consistent values within the ranges set by the Codex Alimentations Commission standard (Commission, 2001).

### 3.8. Fructose/glucose (F/G) ratio

The estimated fructose/glucose (F/G) ratio of honey samples as shown in Table 2 exhibited non-significant difference ($P \leq 0.05$) between Iraqi (1.55 ± 0.08), Turkish (1.69 ± 0.09) and Iranian (1.36 ± 0.07) honey samples, while there was a significant increase as compared with Saudi honey sample. The percentage of F/G shows the potential of honey crystallizes, because the glucose is less soluble in water than fructose (Merwe & Stitt, 2010). According (Demir Kanbur et al., 2021), when the F/G ratio is less than 1.0. honey crystallization crystallization occurs faster; when the ratio is greater than 1.0, it is slower. Therefore, the crystallizing of Saudi honey was faster as compared with other types of honey. whereas Turkish sample was the lowest. The present findings were partially consistent with previous study that have been done on by (Baloš et al., 2020).

### 3.9. Hydroxyl-methylfurfuraldehyde (HMF) concentrations

In the current study, the levels of detected HMF, as shown in (Table 2) showed significant increase ($P < 0.0001$) in Iranian (43.12 ± 0.15 mg/kg) and Saudi (30.22 ± 0.18 mg/kg) honeys samples as compared with other honey samples. (Teferi Damto, 2021) reported that the high HMF content indicated that the honey sample either heated or adulterated by processed sugar. Notably, The HMF concentrations in Iraqi, Turkish and Saudi honey samples were within the allowed maximum range of 40 mg/kg, as recommended by the international standard of codex alimentarius. The value of HMF is routinely utilized to assess the freshness of honey, giving information concerning unsuitable processing and/or unsuitable storage(Besir et al., 2021).

## 4. Conclusions

In conclusion, the quality of the honey samples that are accessible has vary in quality on account of several influences such as seasons, packaging and processing conditions, geographical and botanical origins, and storage period.

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