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Physical Chemical Parameters and Phenolic Compounds Content Analysis of Different Imported Honey in Benghazi, Libya

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Abstract:

The present work aimed to evaluate the quality of five honey samples with respect to their physicochemical parameters and phenolic compound contents. The samples included Saudi Arabia, Indian, Argentine, Spanish, and Germany honey. The studied honey samples were collected from local markets in Benghazi city. The moisture; electrical conductivity, optical density, specific gravity, ash content; water insoluble solids, acidity, reducing sugars, sucrose contents, 5-hydroxymethyl-2-furfuraldehyde (HMF) and phenolic compounds contents were analyzed in each one of the honey samples. The rese ware methods recommended by International Honey Commission. The obtained results were compared with the *Libyan Standard Legislation*. Concerning the physiochemical parameters, most honey samples were harmony with the *Libyan Standard Legislation* for honey. The German honey samples showed the highest phenolic compound contents (163.6 ± 2.04 ppm) while the Spanish honey sample showed the lowest value (48.8± 2.47ppm).

Keywords: *Honey, Chemical parameters, physiological parameters*

Introduction:

Honey is a natural product produced by bee workers from nectar or honeydew [1]. This sweet and flavorful product is one of most complex nature foodstuffs. Honey is mainly composed of carbohydrates (there are up to 75% of sugars mainly fructose and glucose) , 0.2-3.0 % proteins that included a number of enzymes (diastase, invertase, glucose oxidase, cataclses, etc.) traces of organic acids (oxalic, lactic, malic, tartaric, citric) and free amino acids (proline, phenyl alanine), hydroxamines; vitamins, pigments, flavonoids, phenolic, minerals, water, pollen and wax [2 and 3]. These trace contents are known to have distinctive nutritional and therapeutic values and hence have varied applications for enhancing the human health and improving immunity [4].

Honey is rich in nutrients, so it is consumed as a high nutritive value food. Honey also has a bactericidal properties. Its anti-bacterial properties are due to its low acidity and low level of hydrogen peroxide release. Honey is used to treat disease such as gastrointestinal disorders cough and sore throats, bronchial asthma, tuberculosis, ulcers, earaches, dizziness, constipation, eczema; measles, and eye diseases. Honey also has potential therapeutic properties in wounds healing. The moisturizing action of honey around a wound facilitates the healing process and high viscosity of honey inhibits infections to penetrate into the body. Honey has a conservation and stimulating properties, it can prevent deteriorative oxidation reactions in food [4-7].

In Libya the local production of honey is about 500,000 kg per annum, and most Libyan honey sold directly to customers. This locally produced honeys brings a premium price, ranging from 17 to 30 US$ per kg. On the other hand, different imported honey sells in grocery stores, for approximately US $ 7 per kg. Libyans prefer to use local honey because of its quality and authenticity [8]. They use local honey in preparing traditional food and a lot of sweet. In addition Libyan people use local honey as a medicine to treat of different diseases and disorders as a popular home remedy [8 and 9].

In Benghazi markets, there are many types of commercially sold honey, available from different countries such as Egypt, Tunisia, Algeria, Arabia Saudi, France, Greece, Spain, Italy, India, Argentinea and Germany. In Despite to the lower price of the imported honey, the Libyan consumers prefer to use local honey. They believe that local honey is superior then the imported one. Therefore, the present study was conducted to investigate the quality of five different honey brands commercially available on Benghazi markets. The physicochemical characteristic of the imported honey samples will be determined and the results will be compared with Libyan Standard Legislation for honey [10] and with physicochemical parameters of some Libyan honey types published in literature [11].

Experimental Part

Sample Collection

Five commercial honey samples of different floral sources were purchased from local market in Benghazi, Libya. The brands of honey are imported from different countries including Arabia Saudi; Argentine; India; Spain and Germany. Two different bottles of each brand were analyzed in a total of 10 samples. The information concerning common names of the analyzed honey, years of production and regional data are shown in Table 1. The samples were left at room temperature (25±2°C) away from light in airtight glass container, until further analysis.

The tests and parameters of honey samples were conducted using standard equipment and materials, provided by the well-known international companies, in Food Analysis Laboratories, Department of Nutrition, Faculty of Public Health, University of Benghazi, Libya and Analytical Chemistry Laboratories in Chemistry Department, Faculty of Science, University of Benghazi, Libya, during March 2014.

Table (1): Flora type and Production data of five different imported honey in Benghazi, Libya

Honey sample	Flora type	Brand, Year of Production
1. Saudi	Multiflora	ALSHIFA, 2013
2. Indian	Multiflora	SMART BEE, 2012
3. Argentine	Multiflora	EBIA, 2013
4. Spanish	Orange	FLORESTA, 2013
5. Germany	Black Forest (Forest trees)	BLACK FOREST HOENY, 2013

Procedure

The samples of honey were analyzed to determine moisture; optical density; electrical conductivity; ash content; pH; total acidity; Hydroxymethyl furfural (HMF); sugar and polypheonls contents. All the chemicals and reagents used in this work were of analytical grade.

Moisture of honey samples was determine according to AOAC method [12] by measuring the refractive index at 20°C of honey sample using a refractometer (*Bellingham and Stanley model Abbe-type refractometer*). Then the corresponding moisture percentage was obtained from the Wedmore`s Table [12].

Color intensity of honey samples was detected by measuring the absorbance without honey dilution at 420 nm, using a single beam Spectrophotometer (*UV-VIS Spectrophotometer*, *SPECORD* 40, *Analytik Jena*, *Germany*)[13].

Electrical conductivity (**EC**) was measured using a conductivity meter and electrical conductive cell (*Conductometer*: *Connet* 2 *Conductivity Meter with ATC-HANNA-Instruments*) for a solution containing 20 g honey sample in 100 mL distilled water. The conductance is read in mS/cm after temperature equilibrium has been reached [14].

Ash content was determined according to the methods of AOAC 1999[12]. 5 g of honey sample was placed in combustion pot which required preheating to darkness with a gas flame to prevent honey foaming. Then the samples were incinerated at high temperature using electrical furnace. After cooling at room temperature, the obtained ash was weighted.

Water insoluble solids were determined by following Sigth N. and Bath P. K. method [13].

pH and total acidity (T.A.) of honey was determined by potentiometric titration method using standard NaOH solution[12]. 5 g of honey is quantitatively transferred to a 50 ml volumetric flask and filled to the mark with water. 25 ml of this solution is pipette into a 250 ml beaker then the **initial pH** is measured using pH-meter (*Ino lab WTW*) equipped with glass combined electrode (*pH-electrode sen Tix* 61-*B*023009*AP*017). The solution is stirred gently, then 10 ml of 0.05 M NaOH solution is added into the beaker. The excess NaOH is titrated with 0.025M solution of H_2SO_4 solution. The volume of neutralization, corresponding to pH 7, is detected from the acid-base titration curve. The volumes of neutralization are used to calculate T.A., which are expressed in milli-equivalents of sodium hydroxide required to neutralize 1 kg of honey (meq/Kg).

Sugar content, involving reducing sugars (glucose, fructose) and apparent sucrose contents, were determined by Layne-Enyon method using the Fehling's reagent [12]. About 5 g of honey sample was transferred to a 250 mL volumetric flask, then the volume is completed with water. 5 mL of standardized Fehling`s solutions A and B were transferred to a 250 mL Erlenmeyer flask containing 7.0 mL of water, then 1 mL of 0.1% aqueous methylene blue solution is added as indicator. The solution is heated to boiling and then titrated with honey sample solution until the blue color disappeared. The titration is performed duplicate and the average volumes is taken.

Sucrose content was determined by transfer 5 mL of honey solution into 250 mL Erlenmeyer flask, then 45 mL of dist. water and 5 mL concentrated HCl are added. The flask is heated in water bath at 71^{o}C. After 5 min the flask is cooled to 20^{o}C during 2 min. The acidic solution is then neutralized using 20% NaOH solution. The volume of the solution is completed to 100 mL with dist. water. The total sugar content was determined by the titration with Fehling`s solution as shown in Layne-Enyon method and the sucrose content was obtained by calculating the difference.

Hydroxymethyl Furfural (HMF) was determined by dissolving 5 g of honey sample in 25 mL dist. water. The obtained solution was treated with a clarifying reagent (0.5 mL of 15% potassium ferrocyanide and 30% Zinc acetate), then the volume was diluted to 50 mL with dist. water. The absorbance of the filtrate is measured at 284 and 336 nm against an aliquot of the filtrate treated with 5 mL 0.20% bisulphite solution. The HMF content (in mg/100 g honey) is calculated using Equation (1) [12].

$$HMF = \left(\frac{A_{284} - A_{336}}{sample\ weight}\right) * 74.87 \qquad \text{Equation (1)}$$

Phenolic compounds Content of honey samples was estimated using a modified spectrophotometric Folin-Ciocalteu (F-C) method [15]. 20 g of honey is dissolved in 100 mL deionized water. 20 µL of the honey solution is pipetted into small tube and diluted with 1.58 mL water and then mixed with 100 µL of F-C reagent (2N reagent available from *sigma-Aldrich*, *Milan*, *Italy*), the content of tube is mixed well using vortex mixer. After 30 min, 300µL of 20% (w/v) sodium bicarbonate solution was added. The reaction was incubate for 30 min at 40°C, then the absorbance was measured at 765nm against blank solution. The total phenolic content of each honey sample is obtained from the calibration curve prepared by standard solution of gallic acid. The total phenolic content is expressed as mg gallic acid/100 g of honey sample.

Results and Discussion

The physical and chemical properties of five honey samples (including, Saudi Argentinean, Indian, German and Spanish) are shown in Tables (2) and (3).

Moisture content of honey samples range from 17.0-18.5%, table (2). The highest values was recorded for Saudi honey and the lowest value was recorded for Argentinean honey. The maximum limit of moisture content allowed by the Libyan Standard Legislation for honey is ≤17% [10]. However, all the honey samples have little high moisture values, except Argentine honey (17%). The moisture content contributes the honey stability against fermentation, spoiling and granulating during storage. It depends on many factors involving time of extraction; degree of maturity; climate conditions and harvest season; the original moisture of the nectar and the storage conditions [13, 16-18].

Table (2): Some physical characteristics of five different imported honey in Benghazi-Libya

Honey sample	Moisture (%)	Total Ash (%)	Water Insoluble solids (%)	Color intensity (AU)	EC (mS/cm)
6. Saudi	18.5	0.19	0.022	0.777	0.37
7. Indian	18.2	0.17	0.04	1.626	0.53
8. Argentine	17.0	0.13	0.045	1.215	0.31
9. Spanish	17.8	0.72	0.045	0.743	0.20
10. Germany	17.7	2.41	0.071	Out of range	4.60
Libyan Standard Legislation	≤ 17%	≤ 1%	≤ 0.1%	-	-

Ash content of the selected honey samples were 2.41% for Germany honey, 0.72% for Spanish honey, 0.19% for Saudi Arabia honey, 0.17% for Indian honey and 0.13% for Argentine honey (table (2)). The ash contents of the selected honey sample were below the limit allowed by Libyan Standard Legislation for honey (≤ 1%) [10] except Germany honey. Ash content of honey gives an indication of purity and the possibility of adulteration of honey with molasses. It also depends on mineral contents and the materials collected by the worker bees [18 and 19].

Water insoluble solids (total colloid) content of honey samples ranged from 0.022% to 0.071%, table (2). All the values of insoluble water solids were below the maximum allowed limit sets by Libyan Standard Legislation (≤0.1%)[10]. The values of the insoluble water solids content indicate the cleanness of the honey samples. However, the insoluble water solids content represents the suspended wax particles, insect and vegetable debris of honey [20 and 21].

Color intensity of the honey are detected by measuring the absorbance of sample at λ_{420} nm[13]. The results of measuring color intensity show that the Spanish sample has the lowest color intensity value (0.7431AU), table (2). While Germany honey sample has the highest color intensity (out of instrument reading range), this sample has nearly black color. The high color intensity values indicate that the honey sample has high colored contents and pigments such as carotenoids; flavinoids and phenolic compounds [22 and 23]. However, these compounds reflect the antioxidant properties of honey. Also honey color depends on the botanical origin; the amount of suspended particles such as pollen; ash content; exposure to light and the way of handling the combs such use of high temperature or use of old wax combs, for producing honey[24].

Electrical conductivity measurement is used now in routine control of honey sample, instead of determination of ash content. EC is also a good measure criterion index of the botanical origin of honey [25]. EC values of the selected honey samples are shown in table (2). The Germany sample shows the highest value of EC (4.6 mS/cm), followed by Indian and Saudi samples with corresponding values 0.53 and 0.37 mS/cm respectively. Then come Argentinean and Spanish honey with the corresponding EC values 0.31 and 0.20 mS/cm respectively. However, all the samples were with the *European Standard Limit* (Not more than 0.8 mS/cm) [26], except Germany sample. However, the values of conductivity of honey depend on organic acids; proteins; complexes sugars and polyols contents in addition to minerals [27].

Table (3): Analysis of acidity, HMF and sugar contents of five different imported honey in Benghazi, Libya

Honey sample	pH	T. A (meq/kg)	HMF (mg/kg)	Reduced sugar (%)	Sucrose (%)
1. Saudi	3.34	26.0	23.8	76.0	4.1
2. Indian	3.76	30.0	34.8	73.0	4.9
3. Argentine	3.63	18.0	26.0	80.5	1.9
4. Spanish	3.53	12.0	12.9	78.5	2.9
5. Germany	5.05	22.33	25.0	75.5	3.1
Libyan Standard Legislation	acidic	<50 meq/Kg	40 mg/kg	> 65%	<5%

pH values of the selected honey samples are acidic and range from 5.05 to 3.34, as shown in table (3). Among the honey samples Saudi honey is the most acidic (pH 3.34) followed by Spanish (pH 3.53), Argentinean (pH 3.63) and then Indian (pH 3.76) honey. The lowest acidic is detected in Germany honey sample (pH 5.05). All the pH values agree with the Libyan Standard Legislation for honey which requiring the pH value for flora honey to be acidic only [10]. Although Libyan Standard Legislation lacks the specification of pH values of honey, all the samples are within the allowed limit established

European Standards for honey (3.0-5.6) [26]. However, pH of honey is a measure of lactones in honey and pH is a useful index of possible microbial growth. Low pH inhibits the presence and growth of microorganisms [28, 29].

Total acidity content of honey is due to the presence of organic acids, particularly gluconic acid, in equilibrium with their lactones and esters and inorganic acids such as phosphate and chloride [30]. In this study, the highest value of T.A. was recorded for Indian honey (30.0 meq/kg), followed by Saudi honey (26.0 meq/kg), Germany (22.3 meq/kg), Argentinean honey (18.0 meq/kg), then Spanish honey (12.0 meq/kg), table (3). The T.A. content varied significantly among the honey samples which may due to variation in harvest season, flora type or mineral content [28-31]. But none of the honey samples have T.A. values exceed the allowed limit established by Libyan standard Legislation (50 meq/kg) [10].

HMF in honey results from dehydration process of hexose sugars (fructose) catalyzed by acid. HMF provides an indication of the freshness of honey, overheating and storage in poor conditions [2, 32]. In this study, honey samples show HMF content with a maximum value of 34.8 mg/kg for Indian honey. Spanish honey shows the lowest HMF content corresponding to 12.9 mg/kg, table (3). The variation in HMF content of the honey samples may be attributed to different factors including pH; flora source; storage conditions and variation of climate of region from where honey had been extracted [33]. Although HMF content varied among the selected honey samples, all the honey samples have HMF content values below the allowable limit sets by *Libyan Standard Legislation* (40 mg/kg) [10].

Sugars content represents the most important portion of honey composition, mainly fructose and glucose (reducing sugars). In fact, honey contains other sugars such as saccharose (sucrose); maltose; trehalose; melizitose and others [34]. In this study, the reduced sugar content of the selected honey samples ranged from 80.5% to 73.0%, table (3). The Argentinean and Spanish honey samples have the highest percentage of reducing sugars, corresponding to 80.5% and 78.5%, respectively. All the reducing sugar content values of the honey samples are agree with the Libyan Standard Legislation that should be ≥65% [10]. In the other hand, the high sucrose content indicates that honey is early harvesting or adulteration of honey with sugar syrup [4 and 36]. The non-reducing sugar contents of the honey sample under investigation are varied between 4.9%- 1.9%, Table (3). The Libyan Standard Legislation sets a maximum allowable limit ≤5% for sucrose content [10]. All the honey samples are accepted by Libyan Standard Legislation.

In this study, **the total phenolic compounds content** were recorded at 48.8 mg/gallic acid/100g of Spanish honey followed by 80.2 mg/gallic acid/100g of Saudi honey, 83.5 mg/100g for Argentinean honey, 90.8 mg/100g for Indian honey, and then 163.6 mg/100g for Germany honey, Table (4). Those values indicate the high antioxidant activity of the honey samples. The Germany honey sample, which had the dark color, has been reported to have the highest total phenolic compounds content and consequently the highest antioxidant capacity. The amount and types of polyphenolic compounds in honey are variable and depend on the floral origin [36]. However, these values of total phenolic compounds contents were similar to the values reported by Ahmida *et al.* for some types of Libyan honey (32.2-119.4 mg/100g) [11].

Table (4): Total phenolic compounds content of five different imported honey in Benghazi, Libya

Honey samples	Total Phenolic compounds (mg Gallic Acid/100g honey sample)
1. Saudi	80.2± 1.77
2. Indian	90.8± 2.47
3. Argentine	83.5± 1.41
4. Spanish	48.8± 2.47
5. Germany	163.6± 2.04

Conclusion

In this study, the some physicochemical parameters and phenolic compounds content of five imported honey samples were collected from the local market in Benghazi, Libya. The reason of found that the physicochemical parameters of the honey samples were within the limits set by the Libyan Standard Legislation for honey, with a slightly deviated with a parameters like moisture content (most samples) and total ash content (Germany honey). The obtained results indicated that the analyzed honey samples were of good quality. They had acceptable values for maturity, purity and cleanness. Also, the obtained results indicated that the imported honey samples were rich in phenolic compounds content, which will increase their antioxidant activity and their therapeutic value.

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