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Original Article

Physicochemical Characteristics and Total Phenolic Compounds Contents of Libyan Honey from Various Floral Origins

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ABSTRACT

Objective: The aim of this study was evaluation of some physicochemical parameters and total phenolic compounds content of five Libyan honey samples that most commonly consumed in east Libya, Libya.
Methods: Libyan honey samples included mono-floral (*ziziphus louts*; *thymus capitatus*; *eucalyptus*; *Arbutus pavari*) and multi-floral honey, were collected from local market in Benghazi city. The physicochemical parameters of all honey samples, including moisture content; ash content; electrical conductivity; optical density; pH; total acidity, free acidity, sugar contents and hydroxmethylfurfuarl, were measured.
Results and Discussion: Some honey samples showed higher values of moisture content than the maximum permitted level of Libyan standard Legislation, but moisture content values were within the Codex Standard limit. However, the values of the other physicochemical parameters of Libyan honey samples were found to meet the Libyan Standard Legislation for honey. The total phenolic compounds content of the samples ranged from 97.67-123.50 mg gallic acid/100g of honey, with a mean value 109.64 ±11.93 mg gallic acid/100g.
Conclusion: The values of physicochemical parameters of Libyan honey samples varied bases on the floral source, handling and storage condition. The high total phenolic compounds content indicates that the Libyan honey has a high antioxidant potential, especially *Arbutus pavari* honey.
Key Words: Libyan honey, physiochemical parameters, Total phenolic content, *ziziphus louts*; *thymus capitatus*; *Arbutus pavari honey*

1. INTRODUCTION

Codex Alimentarius Commission defined Honey as a sweet and flavorful natural product produced by honey bees. Honey is formed from nectar of plant and sweet deposits gather from various floral sources, modified, transformed by combining with specific substances of honey bees, dehydrate, stored in honeycombs to ripen and mature [1]. This

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sticky and viscous solution is an important energy food and has different medicinal uses. Also Honey and beeswax are used in the beauty industry as a skin moisturizer, softener and to heal the skin tissue [2, 3].

Honey consists of different components, some of its components are existed due to maturation of honey, some are added by the bees and some of them are derived from the plants [4]. The most important components of honey including carbohydrates (mainly fructose and glucose), water, organic acids (oxalic, lactic, malic, tartaric and citric acids), proteins, traces quantities of amino acids (proline and phenylalanine), different enzymes, vitamins, minerals, microelements, flavonoid and phenolic contents [2, 5]. Although the major constituents of honey are nearly the same in all honey samples, the chemical composition and physical properties of natural honey differ according to floral sources on which the bees forage [6]. Furthermore, difference in climate conditions, vegetation or harvesting seasons are important factors that can affect the various properties of honey [2, 3].

Several types of honey are produced in Libya. The main unifloral Libyan honey are *Acacia spp., Arbutus pavati, Cynara Cyrenaica, Arum cyrenaicum, Pinus spp, Cupressus spp, Thymus vulgaris, Lantana camara, Hisbiscus rosa-sinensis, Eucalyptus cawaldulensis, Medicago sativa, Ziziphus lotus, Ceratania siliqua, Cynara (Linnaeus)*. There is a negligible scientific literature on the properties and qualities of Libyan honey [7]. This paper aims to investigate the quality of five brands of the most popular Libyan honey available in local markets in Benghazi. Some physicochemical parameters and the total phenolic compounds content of different types of mono-floral honey, including (*ziziphus louts; thymus ccapitatus; eucalyptus; Arbutus pavari*) and multi floral honey samples have determined. The obtained results will compare with the values of Libyan Standard Legislation for honey and the values reported by *Ahmida et. al 2013* for several types of genuine Libyan honey. The analysis values of Libyan honey samples will also compare with the previously reported values for honey from other countries.

2. MATERIALS AND METHODS

2.1 Sample collection

Five brands of most popular Libyan honey were purchased from local market in Benghazi, Libya. These brands included four unifloral honey (*ziziphus louts; thymus ccapitatus; eucalyptus; Arbutus pavari*) and one multifloral honey. Two different bottles of each brands were analysed in a total of 10 samples. All the samples were produced during 2013-2014 season. The information concerning botanical data, location; local name were recorded in table 1. The samples were left at room temperature (25±2^{o}C) away from light in airtight glass container, until further analysis.

The tests and parameters of honey samples were conducted using standard equipments 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: Types and Regional data of the Some Libyan Honey Samples

Brand/Code	Honey type/flora source	Local Name	Period of Production
Sample 1	*Ziziphus louts*	*Sider*	July 2013
Sample 2	*Thymus capitatus*	*Zater*	July 2013
Sample 3	*Eucalyptus*	*Kafor*	August 2013
Sample 4	*Arbutus pavari*	*Hanone*	September 2013
Sample 5	*Multiflora*	*AlRabia*	March 2013

2.2 Procedure:

The samples of honey were analysed 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.

2.2.1 Moisture (water content) of honey samples was determine according to AOAC method [8] by measuring the refractive index at 20^{o}C of honey sample using Bellingham and Stanley model Abbe-type refractometer. Then the corresponding moisture percentage was obtained from the Wetmore's table.

2.2.2 The optical density of the 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*) [9].

2.2.3 The 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 [10].

2.2.4 The ash content was determined according to the methods of AOAC 2005 (8). 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 weight.

2.2.5 pH, The free acidity (F. A.) and total acidity (T. A.) of honey was determined by potentiometric titration method using 0.1 N NaOH (8). 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 measure 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 and then titrated first with 0.05M NaOH solution until become pH 8.3. Then 0.05 M NaOH solution is added up to

10 mL into the same 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 calculated F. A and T. A, which are expressed in milliequivalents of sodium hydroxide required to neutralize 1 kg of honey (meq/Kg).

2.2.6 Sugar content: reducing sugars (glucose, fructose) and apparent sucrose contents were determined by Layne-Enyon method using the Fehling's reagent [8]. About 5 g of honey sample was transferred to a 250mL volumetric flask, and 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 are taken.

2.2.7 Sucrose content was determined by transfer 5 mL of honey solution into 250 mL Erlenmeyer flask, and then 45 mL of dist water and 5 mL conc HCl are added. The flask is heated in water bath at 71°C. After 5 min the flask is cooled to 20°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 was determined by the titration with Fehling`s solution as shown in Layne-Enyon method and the sucrose content was obtained by the difference [11, 12].

2.2.8 Hydroxymethyl furfural (HMF) was determined by dissolving 5 g of honey sample in 25 mL distilled 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 filtrated 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) (8)

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

Equation (1)

2.2.9 The content of phenolic compounds in honey samples was estimated using a modified spectrophotometric Folin-Ciocalteu (F-C) method [5, 13, 14]. 20 g of honey is dissolved in 100 mL deionized water. 20 µL of the honey solution is pipette 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, 300 µL of 20% (w/v) sodium bicarbonate solution was added. The reaction was incubating for 30 min at 40°C, and then the absorbance was measured at 765 nm 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.

3. RESULTS AND DISCUSSION

Several physicochemical parameters for five different Libyan honey samples were analysed. These physicochemical parameters of honey samples included moisture content; EC; total ash; optical density; pH, T.A; F. A; HMF; reduced sugar; sucrose and total phenolic compounds contents. The results of these parameters are recorded in table (2), table (3) and table (4). The obtained results can be discussed as following:

3.1 Moisture content is the major parameter of honey sample, which will yield the amount of adulteration present in sample easily. A lower moisture content is desirable as it contributes to honey ability to resist fermentation and granulation during storage, thus promoting a longer shelf life [15].

Table 2: Some Physical Parameters of Some Libyan Honey Samples

Brand	Moisture (%)	EC (mS/cm)	Total Ash (%)	Optical density (AU)	pH
Sample 1-*Ziziphus louts*	17.2 ±0.30	0.240±0.07	0.092 ±0.05	2.178±0.667	3.71±0.412
Sample 2- *Thymus capitatus*	18.9 ± 0.99	0.221±0.019	0.051±0.013	1.618±0.212	4.28±0.339
Sample 3- *Eucalyptus*	19.1± 2.12	0.234±0.010	0.129± 0.034	1.549±0.069	4.18±0.496
Sample 4- *Arbutus pavari*	17.6±0.21	0.253±0.02	0.248± 0.057	Out of range	4.45±0.22
Sample 5- *Multiflora*	17.8±0.5	0.237±0.012	0.091± 0.08	2.113±0.501	3.82±0.11
Range	17.2-19.1	0.221-0.253	0.051-0.248	1.549-> 3.00	3.71-4.45
Mean ±s.d	18.12±0.83	0.237±0.012	0.122±0.076	-	4.09±0.313
Libyan Standard Legislation	≤ 17%	-	≤ 1%	-	acidic

EC= Electrical Conductivity, Au= Absorbance Unite

As shown in table 2, the moisture content of Libyan honey samples ranges from 17.2 % to 19.1 %. All the samples had moisture contents higher than the maximum limit sets by Libyan Standard Legislation for honey [16] (≤17%). In the same time, these values of moisture contents are below 21%, which is the maximum limit sets by international regulations for honey [1, 17]. The moisture content values of the investigated samples were slightly higher than the previously reported values of some Libyan honey published by Ahmida et al 2013, where the corresponding values ranges from 13.3-17.2% [6]. This variation in moisture contents may due to many factors, involving in ripening process, including whether conditions; harvest seasons; original moisture of nectar; the conditions of storage and degree of maturity [18]. Furthermore, the moisture content values of Libyan honey samples under investigation were similar to those values reported for Saudi honey (13.5-18.2%) [19] and Tunisian honey 17.27-19.80% [20].

3.2 Total Ash of honey samples varied from 0.051% to 0.248%, table 2. All the ash content values were within the limit sets by Libyan Standard Legislation for honey (≤ 1%) [16]. However, This range is less than the ash content range recorded by Ahmida *et al* 2013 for some Libyan honey (0.14-0.43%) [6]. In fact, the total ash content of honey depends on the materials collected by bees foraging on the floral [9, 10].

3.3 Electrical conductivity values of the investigated honey samples ranges 0.221-0.253 mS/cm, table 2. Although the EC properties has not legist by Libyan Standard Legislation for honey [16], all EC values are less than the limit sets by European legislation for honey (0.8mS/cm) [1]. However, EC property was recently included in the international standard, replacing the determination of total ash content [1]. In fact our results demonstrated that there is a good correlation between EC and total ash values [21].

3.4 Optical density of the Libyan honey samples varied from 1.589 AU to > 3.00 AU, table (2). However, Among Libyan honey *samples, sample 4 (Arbutus pavari* honey sample) has the highest optical density value. The optical density of the honey is usually correlated with the phenolic and flavonoid compounds contents of the honey [5]. However, *Arbutus pavari* honey sample has the highest value of phenolic compounds content, table 3.

3.5 pH values indicate the freshness of honey and has a great importance during honey extraction and storage, due to influence on texture, stability and endurance [22]. All the analysed honey samples were acidic with a pH in range of 3.71-4.45, table 3. This range is similar to the range that reported for some Libya honey (3.56-4.99) [6], Algerian honey (3.70- 4.00) [23] and Saudi honey (3.5-5.00) [19]. As maximum limit of pH values of honey sets between 3.4-6.1 [23], therefore all of Libyan honey samples full within this regulation [1].

Table 3: Some Chemical Parameters of Some Libyan Honey Samples

Brand	Total Acidity (meq/Kg)	Free Acidity (meq/Kg)	HMF (mg/Kg)	Reduced Sugar (%)	Sucrose (%)
Sample 1-*Ziziphus louts*	20.5 ±0.233	14.17±5.90	20.0 ±1	79.90±1.20	2.78±0.20
Sample 2- *Thymus capitatus*	17.0 ± 0.90	16.67±1.20	28.5±0.71	77.24±1.76	3.85±0.35
Sample 3-*Eucalyptus*	20.67± 2.12	18.67±0.010	30.0± 7.07	76.5±3.68	3.95±1.34
Sample 4- *Arbutus pavari*	18.67±0.21	14.0±1.20	39.0± 0.50	72.63±2.06	5.0±0.46
Sample 5-*Multiflora*	18.33±1.85	15.11±1.58	19.67± 3.5	78.28±1.64	2.62±0.73
Range	17.0-20.67	14.0-18.67	19.67-39	72.63-79.90	2.62-5.0
Mean ±s.d	19.03±1.55	15.88±2.23	27.43±8.02	76.57±3.00	3.64±0.97
Libyan Standard Legislation	≤40meq/Kg	-	≤ 40meq/Kg	≥ 65%	≤ 5%

3.6 Total acidity and free acidity

The acidity of honey is due to the presence of organic acids (e.g gluconic acid) and inorganic ions (e.g phosphate; sulphate and chloride ions) [24-26]. The values of free acidity and total acidity of Libyan honey samples under investigation varied from 14.0-18.67 meq/Kg and 17.0-20.67 meq/Kg respectively, as shown in table 3. None of the samples exceeded the highest limit sets for total acidity by Libyan Standard Legislation (≤ 40meq/Kg) [16]. However, this range of total acidity is slightly different from that range previously reported by Ahmida *et. al* 2013 (10.29-24 meq/Kg) for genuine Libyan honey samples [6]. This difference in the total acidity values of Libyan honey may due to differing in harvesting seasons and flora types [26, 27]. However, Eucalyptus honey sample had the highest total acidity value (18.67meq/Kg). This value is similar to the acidity value reported by Owayss in 2005 [28].

3.7 Sugar content, in this work the reduced sugar content of the honey samples varied from 72.63% to 79.90 %, with a mean value corresponding to 76.57±3.0, as shown in table 3. Sample 1 (*Ziziphus louts* honey) had the highest reduced sugar content, while sample 4 (*Arbutus pavari* honey) had the lowest value, table (3). All the samples have a percentage of reduced sugar content above 65%, the minimum limit sets by Libyan Standard Legislation [16]. The mean value of the reduced sugar content of Libyan honey are similar to those values reported for Egyptian (69.84%±0.31) [29], Yeman (64.21%±0.15) [29], Saudi honey (55.2 %-73.7%) [26], Algerian honey (60.19% -67.7%) [23] and Tunisian honey (67.56-74.31) [20].

Furthermore, the sucrose contents of Libyan honey samples vary from 2.62-5.0%, table (3). All the value of sucrose contents of honey samples were accepted by Libyan Standard Legislation and international regulation for sucrose content in honey [1, 16], which sets at ≤ 5% [1, 16]. Sample 4 (*Arbutus pavari* honey) had the highest content of sucrose. However, the high sucrose content of honey indicates early harvesting of honey or over fed of honeybees with sugar syrup [30, 31].

3.8 Hydroymethyl furfural

The freshness of honey is mainly detected by the determining the concentration of HMF [22]. With the exception of a single sample that contained 39.0 mg/Kg of HMF, the concentration of HMF in the remaining honey samples, ranges from 16-28 mg/Kg, as shown in table 3. All the values are within the permitted limit sets by Libyan Standard Legislation [16]. HMF contents values of the investigated Libyan honey samples were higher than previously reported by Ahmida *et. al* 2013 (1.89-8.24mg/Kg) [6]. The high HMF content may be due to high sugar content of the investigated honey samples. The age of honey and floral source can also influence HMF contents. Another factor that affects HMF content of the honey is hot weather. In fact honey produced in tropical and subtropical climates has HMF contents exceeding 40 mg/Kg [32, 33]. The HMF contents of the investigated honey samples were similar to the values of neighboring countries such as: Algeria (15-24.21mg/Kg) [23], Morocco (0.09-53.38 mg/Kg) [34], Egypt (2.21-23g/Kg) [29], Saudi (10.09-31.55mg/Kg) [19].

3.9 Phenolic Compounds content

An interest in determining the total phenolic compounds content of honey is increasing in recent years [6, 14, 26]. It has been reported that phenolic compounds are correlated with the antioxidant activities of honey [35]

The total phenolic compounds contents of the investigated honey samples were in the range 97.67-123.50 mg gallic acid/100g with an average 109.64±11.93 mg gallic acid/100g, table 4. These values and average of phenolic compounds contents of Libyan honey were similar to the other values reported for some Tunsian honey (32.17-119.42 mg gallic acid/100g) [20], Black forest honey (89.91±11.75 mg gallic acid/100g) [26], Thai honey (100-144 mg/100g) [36] and Venezuelan honey (93.50±51.62) [26]. It has been reported that the amount and types of polyphenolic compounds in honey, and consequently antioxidant activities, are variable and strongly correlated with the floral origin and color of the honey [37]. However, sample 4 (*Arbutus pavari* honey) exhibited the highest phenolic compounds content (123.5 mg gallic acid/ 100g). As shown in table 2, this sample also shows the highest optical density value, which may contributes to the high content of phenolic compounds content. [38]

Table 4: Total Phenolic Compounds content of some Libyan Honey Samples

Brand	Total Phenolic Compounds Content (mg/100g)
Sample 1-*Ziziphus louts*	99.2±8.66
Sample 2- *Thymus capitatus*	120.56±3.96
Sample 3-*Eucalyptus*	97.67±14.14
Sample 4- *Arbutus pavari*	123.5±0.816
Sample 5-*Multiflora*	107.27±7.77
Range	97.67-123.5
Mean ±s.d	109.64±11.93
Libyan Standard Legislation	-

4. CONCLUSION

This study has shown that the investigated Libyan honey samples are of a good quality in respect to physicochemical parameters like ash content, EC, pH, T. C. and F. C. , reduced sugar, sucrose contents and HMF. All values of these parameters were with the permitted limits set by Libyan standard Legislations or international regulations. Libyan honey samples had slightly high levels of moisture content than the maximum permitted limit sets by Libyan Standard legislation but in the same time less than the maximum limit sets by international regulations. The results of physicochemical parameters have established that Libyan honey samples to be fresh and safe for consumption.

The honey samples have high total phenolic compounds contents which indicate good antioxidant properties. *Arbutus pavari* honey sample which has the high optical density value contained the highest content of total phenolic compounds.

Further researches on other hidden properties, such as the content of proteins, enzymes, vitamins essential and non essential elements, are recommended.

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