Analysis of Trace and Essential Elements, Phenolic and Flavonoid Compounds in Libyan Honey Samples Collected from Misurata Markets

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Abstract

Honey is a popular natural food product with a very complex composition comprising both organic and inorganic constituents. The composition of particular honey is strongly influenced by both natural and anthropogenic factors, which vary based on its botanical and geographical origins. It is observed that although the honey which are produced in Libya are of a good quality, it does contain heavy metals. The contents of the metals found in Libyan honey samples were found to be between 465.1-792 mg/kg for Na, 1100.8-1781.6 mg/kg for K, 927.79-1186.6 mg/km for Ca, 0.0033- 0.0177 mg/kg for Cd, 0.0029- 0.0241mg/kg for Pb, 0.0012-0.0042mg/kg for Cr, 0.004-0.032 mg/kg for Cu, 0.72-1.37 mg/kg for Ni and 3.677-7.430 mg/kg for Zn. These results indicated that the quality of the Libyan honey samples tested was very good and matched the world standard properties of honey. Also, the Libyan honey samples examined have a high level of phenolic (792- 99 mg/kg) and flavonoid (1012-687mg/kg) compounds compared with other honey.

Keywords: Libyan Honey, Heavy Metal, Flavonoid Compounds, Phenolic Compounds, Misurata

1. Introduction

[1.] Honey is a natural product produced by honey bees (*Apis mellifera*) that collect the nectar from flowers and convert it into a delicious food product that is known to be a better nutritional option to plain sugar [1]. Honey also contains minerals and heavy metals which play important roles in determining its quality. The mineral content varies, ranging from 0.04% in pale honey to 0.20% in darker honey [2]. Since honey bees can fly up to 4 km in all directions from their apiary, thus having access to a foraging area of about 50 km^2 [3], and the bees come in contact not only with the atmosphere but also with soil and water, the concentration of heavy metals in honey can reflect their presence in the region [4]. The major minerals in honey are mainly derived from the soil and nectar-producing plants, but they may also appear from anthropogenic sources, such as environmental pollution. It has been reported that micro- or trace minerals originating from organic or plant sources are important for maintaining human health, while those which originate from inorganic or metallic sources, such as heavy metals, can be toxic to humans [5,6]. Reports indicate that honey samples collected in industrial regions had higher heavy metal content (Cd, Pb, Hg, Zn, Cu, Ni and Cr) than those obtained from rural areas. The accumulation of toxic metals in the human body causes side effects that are detrimental to health, so the determination of heavy metal content in honey is a critical factor for human nutrition and safety [7]. Plant polyphenols are primarily natural antioxidants having several beneficial functions as reducing agents, metal chelators, and singlet oxygen quenchers [8]. Flavonoids and simple phenolic derivatives such as phenolic acids comprise the majority of plant polyphenols and are considered as the main source of antioxidants in honey [9]. The average antioxidant contents of honey have been determined and found to correlate with their phenolic content [10]. Polyphenols have also been reported to affect the flavor [11] and the physical appearance of honey, particularly the honey color [12]. Bertoncelj et al. [13] reported a positive correlation between the phenolic content, coloring, and antioxidant activity in honey. Flavonoid profiles in honey generally vary widely and they differ according to their floral and geographical origins [14]. Also, Maurya et al. [15] have stated that the botanical origin of honey has the greatest influence on its antioxidant and phenolic content while processing handling and storage affect honey only to a minor degree. Our objectives in this study were to analyze nine metallic elements and the phenolic and flavonoid content of ten honey samples from five honey producing regions in Libya.

1. Material and Methods

2.1 Sampling

Ten different honey samples obtained from a local market in Misurata, Libya and seven samples were taken from each honey as described in Table1. The honey samples collected were labeled (H1 - H10) for ease of identification. All samples were stored in glass jars and kept under refrigeration at 4 °C until needed for the different analyses. All chemicals used were of analytical reagent grade.

Table (1). Ten types of Libyan honey samples

Sample code	Local name	Honey type/floral source	Location	Colour (OD)
H1	Elseder	Ziziphus lotus	Valley of Sasso Misurata	0.867
H2	Kasool	Mesembryanthemum	Alzarrog Misurata	0.809
H3	Bersim	Trefoil	Alseket Misurata	0.921
H4	Elateal	Tamarix aphylla	Al- Jufra	0.937
H5	Elrabee	Multiflora	Alseket Misurata	1.465
H6	Elkafoor	Eucalyptus	Thanh Misurata	1.054
H7	Habet albaraka	Nigella sativa	Valley of Sasso Misurata	1.13
H8	Elzaater	Thymus capitatus	Thomas	0.666
H9	Elkabat	Forest honey	Shahat	1.76
H10	Hazmat	Citrus	Al-Zahra Tripoli	0.958

2.2 Heavy metal analysis of honey samples

Specimens (~ 1.0 g) of honey samples were weighed accurately in a covered vessel and 6.0 ml of HNO_3 (65%) were added. The mixture was allowed to stand at room temperature until the initial reaction had subsided and then 2.0 ml of H_2O_2 (30%) were also added. The mixture was then transferred to a microwave unit (MARS6 Microwave Digestion- CEM Microwave Technology Ltd) and then the microwave program was started, setting the appropriate time and wattage. The vessel was cooled, and the contents carefully rinsed with deionized water three times, transferred to a beaker, and filtered into a 25.0 ml volumetric flask and made up to the mark with deionized water. The toxic heavy metals concentrations were evaluated using an atomic absorption spectrometer (Hitachi 30-180 Spectrophotometer; Hitachi Ltd, Japan) fitted with a hollow cathode lamp. Blank standard heavy metal solutions of 1000 ppm concentration were diluted accurately to 3, 2.5, 2, 1.5, 1.0, 0.5, 0.1, 0,05 ppm concentrations and aspirated before the analysis of the digested solutions of honey samples was carried out using AOAC methods [16].

2.3 Quality Control

Quality control of this test procedure was assured by using certified reference materials. Analyses of certified reference materials allowed an assessment of accuracy and precision over a range of element concentrations to be made. The observed values correlated well with the certified values as shown in Table 2. The accuracy of the microwave digestion method was checked using the standard reference materials. Three replicates were taken for each sample of certified reference material and two measurements were performed for each digested sample used.

Table 2. Trace and Essential elements concentrations in certified reference material

Element	Certified data (ppm)	Experimental data (ppm)	Recovery (%)
Na	10	9.97	99.7
K	10	9.98	99.8
Ca	10	9.96	99.6
Cu	10	9.98	99.8
Cr	10	9.95	99.5
Cd	10	9.98	99.8
Pb	10	10.1	101
Zn	10	9.97	99.7
Ni	10	9.93	99.3

2.4 Total phenolics

The total phenolic content of the honey samples was measured using the Folin-Ciocalteu method according to Socha et al., [17] and Ferreira et al., [18]. The honey stock solutions were prepared at a concentration of 0.04 g/ml of distilled water. A 0.5 ml aliquot of the stock solution was mixed with 0.3 ml of the Folin-Ciocalteu reagent followed by the addition of 2 ml of a 15% solution of sodium carbonate. Distilled water was added to make up to 5 ml and the mixture was incubated for 2 hrs. The absorbance of the mixture was measured at a wavelength of 798 nm. A standard curve for gallic acid was prepared for quantification calibration, using a concentration range between 8 and 40 mg/l, and the results were expressed as mg gallic acid/kg honey.

2.5 Total flavonoids

The total flavonoid content in the honey samples was determined according to the method of Zhishen et al., [19]. One ml of the diluted honey specimen (0.2 g/ml) was mixed with 4 ml of distilled water followed by the addition of 0.3 ml of 5% sodium nitrite solution. After 5 min, 0.3 ml of 10% aluminum chloride solution was added, and six minutes later 2 ml of 1M of sodium hydroxide solution was added and the volume was made up to 10 ml using distilled water. The mixture was shaken and the absorbance was measured at 510 nm using a spectrophotometer. A calibration curve was made using a standard solution of catechin over the concentration range 20–100 mg/l. The results were presented as mg of catechin equivalents per kg of honey.

2. Results and Discussion

3.1 Metal Content Analysis

Average metal concentrations in the honey samples are given with their standard deviations in Tables 3, 4. As the Libyan Standard Legislation for honey does not set a maximum limit for the presence of metals in honey, so noticeable contents of heavy metals imply observable contamination with these metals.

Table 3. Average Na, K and Ca concentrations in the honey samples (mg/kg) and their standard deviations

Sample code	**Na**	**K**	**Ca**
H1	702.2 ±7.38	1781.6 ±13.84	1014.7 ±9.16
H2	552.1 ±22.71	1623.1 ±21.32	1067.5 ±8.18
H3	634.1 ±11.21	1372.4±13.24	1164.8 ±20.22
H4	465.1±33.42	1462.5 ±14.96	1186.6 ±19.01
H5	632.4 ±23.14	1466.9±10.16	1183.4 ±10.8
H6	792.3 ±1.16	1189.5 ±9.87	983.46 ±11.3
H7	593.1 ±32.16	1100.8 ±3.01	979.22 ±10.1
H8	645.3 ±2.42	1428.1±11.79	927.79 ±4.43
H9	632.1 ±75.85	1154±23.85	1095.48 ±9.25
H10	719.6 ±2.28	1314.3±21.54	989.26 ±11.95

The study shows that potassium, calcium, and sodium are the most abundant elements in all the honey samples studied. The highest values of potassium, calcium, and sodium content of the honey samples studied here were 1781.6 ±13.84, 1186.6 ±19.01 and 792.3 ±1.16 mg/kg, respectively. Table 2 and Fig.1 show that the major elements occur in the order K > Ca > Na for our honey samples. The high concentration of potassium found in the honey samples can be attributed to the high levels of potassium ions that occur in the plant tissues. Moreover, the differences in metal content may be attributed to the environment and to the soil found in the sample locations. Comparing the values of the essential elements Na, K, and Ca obtained from the honey samples with the values reported earlier by Awad [20], we observed that the range of concentrations of Na from 465.1±33.42 to 792.3 ±1.16 mg/kg, were lower than the values reported by Awad [19], namely 961.0 to 2110 mg/kg. The analogous values for Ca were 927.79 ±4.43 to 1186.6 ±19.01 mg/kg, which are lower than the values reported by Girliane [21] for Brazilian (Gandara) honey 1864 to 3424.9 mg/kg. Saadiyat et al., [22] reported that the potassium content in Brazilian honey samples ranged from 5918.5 to 13366.6 mg/kg. Although described as essential metals, high concentrations of Na, K, and Ca also produce toxic effects when the metal intake is excessively increased.

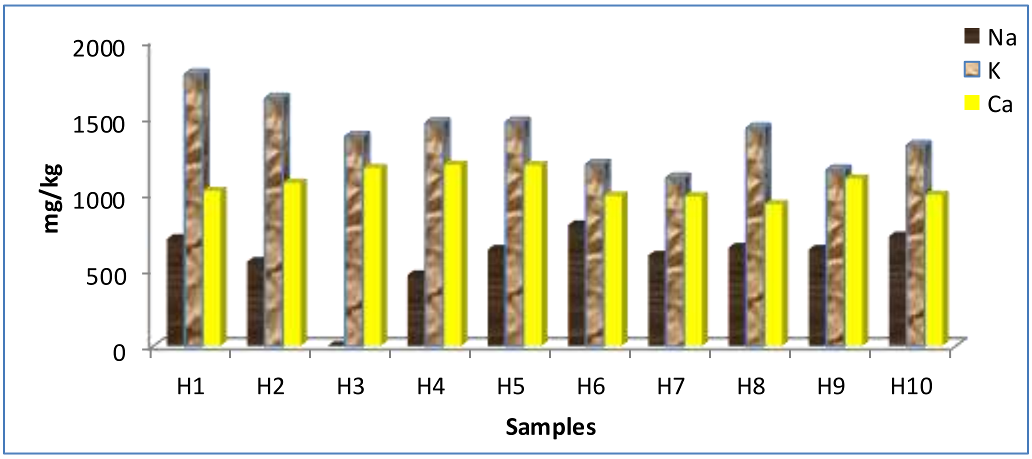

Fig. 1 Distribution of sodium, potassium, and calcium in honey samples

Table 4. Average Cu, Cr, Cd, pb, Ni and Zn concentrations in the honey samples (mg/kg) and their standard deviations

Sample code	Cu	Cr	Cd	Pb	Ni	Zn
H1	0.021±0.008	0.0027±0.0003	0.0115±0.0037	0.0146±0.002	0.96±0.05	3.705±0.01
H2	0.025±0.005	0.0023±0.0002	0.0163±0.0055	0.0043±0.001	0.94±0.01	7.185±0.03
H3	0.017±0.004	0.0020±0.0001	0.0040±0.0012	0.0142±0.002	0.85±0.01	4.833±0.02
H4	0.004±0.001	0.0012±0.0002	0.0033±0.0015	0.0029±0.001	1.11±0.01	6.476±0.06
H5	0.021±0.003	0.0035±0.0003	0.0040±0.0011	0.008±0.001	1.22±0.02	4.885±0.12
H6	0.022±0.006	0.0025±0.0007	0.0177±0.0068	0.009±0.002	0.86±0.05	6.030±0.15
H7	0.032±0.005	0.0042±0.0003	0.0033±0.0015	0.004±0.001	0.72±0.03	7.430±0.13
H8	0.007±0.004	0.0032±0.0004	0.0042±0.0011	0.003±0.001	0.78±0.07	3.677±0.02
H9	0.008±0.001	0.0022±0.0008	0.0033±0.0016	0.003±0.001	0.73±0.06	6.340±0.01
H10	0.006±0.002	0.0027±0.0001	0.0043±0.0005	0.0241±0.005	1.37±0.04	6.94±1.13

From Table 4 and Fig. 2, it can be seen that the mean values of Cd metal concentration in honey samples fall in the range of 0.0033±0.0016 to 0.0177±0.0068 mg/kg. The results show there is no significant difference in the concentration of Cd in any of the samples. These results were lower than those reported by Rashed and Soltan [23] for Egyptian honey, namely 0.1 to 0.5 mg/kg, and Saadiyat et al., [22] for Saudi Arabian honeys, namely 0.1 to 0.16 mg/kg, and are also lower than the recommended value reported by the Standard Codex limit of 0.05 mg/kg [24]. According to the maximum limit for cadmium content set by the European Legislation (0.1 mg/kg), all the Libyan honey samples examined here have much lower values of cadmium metal contamination [25].

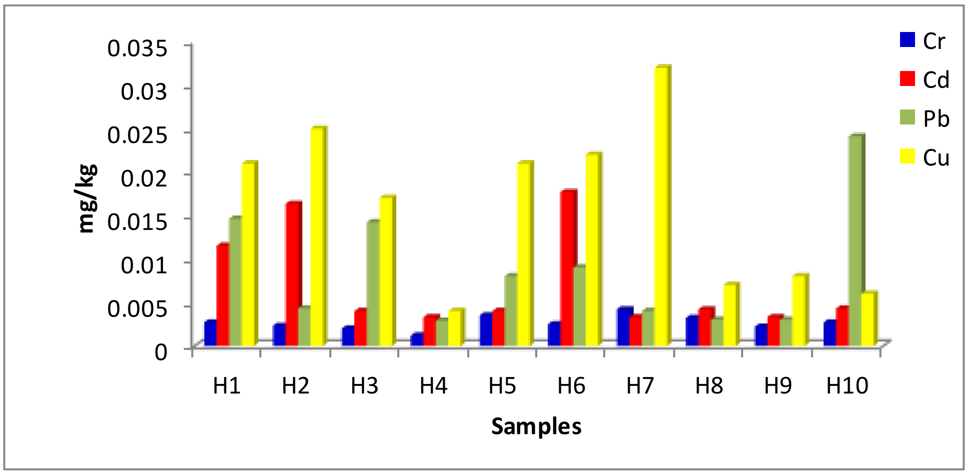

Fig. 2 Distribution of Chromium, Cadmium, Lead and Copper in honey samples

The mean values of lead content in the honey samples studied ranged from 0.0029±0.001to 0.0241±0.005 mg/kg, and were clearly lower than the values reported by the Codex (less than 0.1 mg/kg). However, it is reported that the maximum Pb levels in honey samples are normally 0.5 mg/kg [26], but in the European Union

Member States, the maximum permitted Pb level in honey samples is 1.0 mg/kg [27]. When comparing the mean value range of Pb content determined in the current study with those found in Argentinian, Australian and Brazilian honey, the highest values of Pb concentration appeared in the Argentinian, Australian and Brazilian honey samples, namely 115.17 to 332.33 mg/kg, whilst the Pb values in the Egyptian honeys range from 4.12 to 9.3 mg/kg. Saadiyat et al. [22] reported that the Pb content in Turkish honey ranged from 8.4 to 44.1 mg/kg. Salama et al. reported that the Pb content in honey from the west of Libya ranged from 2.42 to 10.98 mg/kg [28]. A high concentration of Pb ions in honey described in other studies may indicate a possible source ascribed to environmental pollution, as revealed by Lambert et al. [29]. The honey samples contained a chromium concentration from 0.0012±0.0002 to 0.0042±0.0003 mg/kg as shown in Fig. 2, with an average value of 0.00265 mg/kg. Reported chromium concentration values in the literature for honey were 0.010–0.10 mg/kg [30] and 0.043–1.07mg/kg [31]. The values for the Cr content in our samples are generally lower than the levels reported in the literature [30, 32]. Copper is one of the essential metals that may be present in small amounts in honey. It is not toxic if it is present in only a limited amount. Although the Libyan Standard Legislation of honey recommended the absence of copper metal, it was detected in all honey samples tested here. Its concentration ranged from 0.004±0.001mg/kg (sample H4) to 0.032±0.005mg/kg (sample H7). Italian legislation [33] for the presence of copper metal in honey sets a limit at 0.216 mg/kg. According to Italian legislation, all the honey samples under investigation were found to be not contaminated. On the other hand, the Nigerian regulation [34] has set a rather high limit of 35 mg/kg for the presence of copper metal content in honey samples, and this means that the copper metal content of Libyan honey samples is significantly lower than this permissible limit.

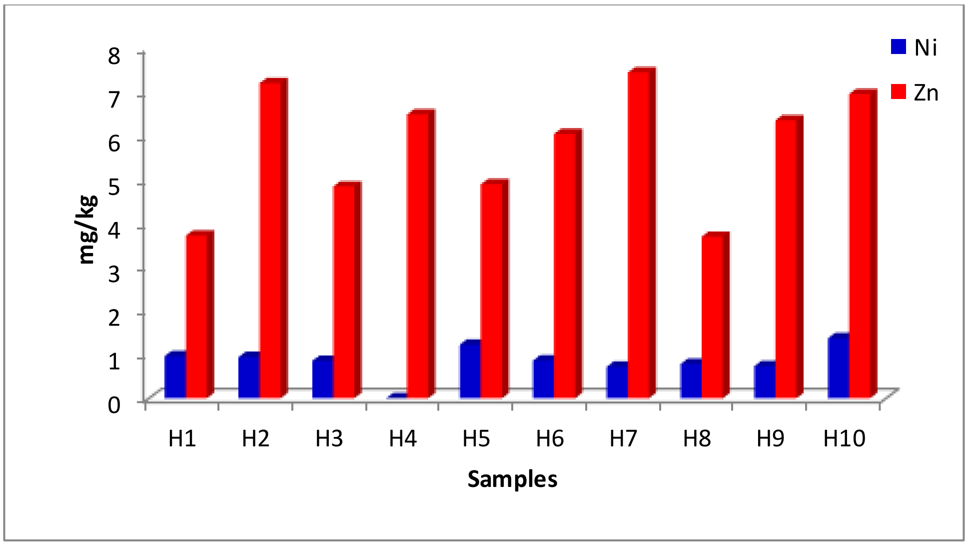

Fig. 3 Distribution of Zinc and Nickel in honey samples

Zn is generally considered to be an antioxidant and is found in nearly 100 specific enzymes. The lowest and the highest Zn concentrations in our specimens were 3.677±0.02 mg/kg in honey sample H8 from Khoms and 7.430±0.13mg/kg in honey sample H7 from Valley of Sasso, Misurata, respectively, as shown in Fig.3. The average value for Zn content of 5.75mg/kg was slightly lower than that found in the analysis of Anatolian honey samples with a mean level of 1.1–12.7 mg/kg [21] and Eastern Slovakian honey samples with a range of 1.59–13.03 mg/kg [8]. Zinc values in honey samples have been reported in the range of 0.18-19.1 mg/kg [39], 1.15-4.95 mg/kg [4], 1.6-22.5 mg/kg [41], and 4.17-22.3 mg/kg [35]. The lowest and highest nickel concentrations in our specimens were found to be 0.72±0.03 mg/kg in the honey sample H7 from the Valley of Sasso, Misurata and 1.37±0.04 mg/kg in the honey sample H10 from Al-Zahra, Tripoli. Nickel values in the literature have been reported in the range 0.23–0.27 mg/kg [40] for honey samples from Italy. The nickel levels in our samples are higher than those reported by Caroli [35] for Italian honey.

Table 5. Total phenolic contents and flavonoids in Libyan honey samples

Sample code	Phenolics (mg/kg)	Flavonoids (mg/kg)
H1	828±1.5	687±1.2
H2	962±3.5	789±3.5
H3	904±1.2	889±1.5
H4	792±4.1	973±2.1
H5	946±2.1	883±1.5
H6	912±2.5	992±3.5
H7	882±1.9	709±1.4
H8	873±5.3	993±1.6
H9	994±2.3	1012±1.1
H10	802±1.8	945±1.5

3.2 Total phenolics

The total content of phenolics ranged between 792±4.1 and 994±2.3 mg/kg as shown in Table 5. Generally, our samples show a higher phenolic content compared with honey from other countries. For example: Malaysian honey 305.5–419.9 mg/kg [39]; Czech honey 39.2–167.1 mg/kg [40]; Portuguese honey 132.2–727.8 mg/kg [18] and Brazilian honey 250–548 mg/kg [41]. The most similar phenolic contents to Libyan honey were reported for Mexican honey, namely, 621.3–1368.4 mg/kg [46]; Moroccan honey, 239.5–1138.5 mg/kg [43] and Argentinian honey 401.8–1188.2 mg/kg [44]. The high phenolic content in the Libyan honey studied here confirmed the good quality of the honey. The variation in the phenolic content for our samples compared favorably with those from different countries and this is dependent upon the different geographical and botanical sources of honey.

3.3 Total flavonoids

The total flavonoids in our samples were highest in the H10 (Al-Zahra Tripoli) sample which averaged 1012±1.1 mg/kg, while in the H1 (Valley of Sasso, Misurata) sample the average was 687±1.2mg/kg. These results were higher than the corresponding values reported for Argentinian honey 69.4–677.6 mg/kg [43]; Portuguese honey 123.6–587.4 mg/kg [18]; Moroccan honey 11.7–179.1 mg/kg [40] and Malaysian honey 135.3–165.3 mg/kg [39]. The higher flavonoids found in our samples compared with others could be due to botanical and geographical differences as well as to climate and environmental factors such as humidity, temperature and soil composition.

3. Conclusions

Ten honey samples were collected from markets in Misurata, Libya. The results of this study clearly show that honey samples collected from eight locations in the west of Libya have good properties and high concentrations of essential elements and may be considered as a useful dietary source of these elements for human consumption when taken freshly in crude form or manufactured in honey products. The Libyan honey studied here compared favorably with honey from other countries and were rich in phenolics and flavonoids, confirming that this honey can be used as natural food ingredients as well as a rich source of antioxidants in the human diet.

4. Conflicts of Interest

The authors declare that they have no conflict of interest regarding the publication of this paper.

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