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Physico-chemical properties of multi-floral honey from the West Bank, Palestine

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ABSTRACT

Honey is a nutritious product that is produced by honey bees. Its nutritional value and genuine quality is of great importance to consumers. The present study aimed at evaluating the physico-chemical properties of Palestinian multi-floral honey from the West Bank. For this purpose, a total of 33 honey samples were collected directly from honey beekeepers in different geographic regions of the West Bank and analyzed for pH, moisture, relative density, total sugars, reducing sugars, sucrose, fructose, proteins, ash, hydroxymethylfurfural, and mineral content. The mean physico-chemical contents were: pH = 3.44, moisture = 16.53%, relative density = 1.424, reducing sugars = 78.86%, sucrose = 4.10%, fructose = 38.29%, proteins = 0.33%, ash = 0.14%, and hydroxymethylfurfural = 12.32 mg/kg. Cd and Pb were below detection limits in all samples analyzed. Honey samples were rich in K, Na, Ca, Pb, and Mg with concentrations ranging between 183.86 mg/kg (K) and 22.74 mg/kg (Mg). Other trace elements (Fe, Li, B, Mn, Ba, Zn, Cu, Cr, and Ni) were found in much smaller concentrations between 5.21 mg/kg (Fe) and 0.08 mg/kg (Ni). In conclusion, the results of physico-chemical analysis of Palestinian multi-floral honey indicated purity and good quality that meet the international honey standards.

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Introduction

Honey is the natural sweet substance processed and produced by honey bees (*Apis mellifera*) from the nectar of plants.[1] It is one of the most important bio-products that is characterized by high nutritional value (330 kcal/100g) and fast absorption of its carbohydrates upon consumption.[2] Besides, honey is considered a medicinal food that has antibacterial properties.[3,4] and can be used as an indicator of environmental pollution.[5,6] The quality of honey is dependent on the type of flowers from which the bees collect the nectar,[7–9] the climatic conditions under which plants grow and the conditions of processing and storage of honey.[10,11] Besides, honey might become contaminated with antibiotics, pesticides, and heavy metals.[12–14] Therefore, monitoring honey quality parameters is of great importance to consumer's health.

Palestine has rich biodiversity because of its geophysical characteristics and climate. Its flora consist of over than 2780 flowering plant species. The floral biodiversity of the country is of great importance to honey production. In 2010, the number of honey bee hives in Palestine mounted 51,698 producing some 500 tons of honey.[15] However, studies that evaluate Palestinian honey quality parameters are almost lacking. Swaileh and Abdulkhaliq[16] analyzed Palestinian honey for the presence of aflatoxin, nicotine, caffeine, and heavy metals. Another study[17] investigated the effect of Palestinian honey on spermatogenesis in rats. Odeh et al.[18] used volatile compounds as markers in Palestinian honey from *Thymus capitatus*, *Thymelaea hirsuta*, and *Tolpis virgate*. Therefore, the present study is considered the first of its kind that aims at evaluating the Palestinian multi-floral honey quality parameters namely sugars content, pH, water content, relative

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density, hydroxymethylfurfural (HMF), ash, proteins, and essential mineral content for honey samples from different geographic regions of the West Bank.

Materials and methods

Honey samples

A total of 33 multi-floral honey samples (1 kg each) were collected from honey beekeepers in the West Bank from April–August of 2009. Honey samples originated from four geographic regions, namely the North West Plains, Jordan Valley, the Central Mountains, and the Southern Semiarid Region. Samples were kept in glass containers in the laboratory under room temperature for later analysis.

Honey quality tests

Honey total sugar content, relative density, and water content were determined using a refractometer REF-116 with ATC (MRC, Israel). Honey pH was measured by a Jenway 3510 pH-meter (Bibby Scientific Ltd., UK) in a solution of 10 g honey dissolved in 75 mL of distilled water (AOAC method 920.181). Fructose, sucrose, and reducing sugar contents of honey were analyzed by Waters 2690 Alliance high-performance liquid chromatography (HPLC; Waters Corporation, USA) using the following HPLC conditions: mobile phase: LC-NH_2 acetonitrile-water (85:15, *v:v*), ultraviolet (UV) photo diode detector set at 196 nm, flow rate of 2.5 mL/min and a Supelcosil™ column 25 cm × 4.6 mm with particle size of 5 µm. The column oven temperature was 45°C, injection rate was 10 µL and run time was 15 min. Honey HMF content was measured according to AOAC 980.23 method using a UV/V is spectrophotometer Lambda 25 (Perkin Elmer, USA). Ash was determined by ashing 10 g of honey at 600°C for 5 h according to the AOAC 920.181 method using Bifatherm Furnace MS 8 (Bifa, UK). Protein content was determined using the Kjeldahl method, which includes digestion with acid, distillation, and titration according to AOAC 962.18 method using Kjeldahl Apparatus, Vapodest 50 (Gerhardt, Germany).

For metal analysis, honey sample were dried and ashed in a Bifatherm MS 8 Furnace (Bifa, UK) at 600°C according to AOAC 920.181A method. Thereafter, the residues were dissolved in concentrated nitric acid and diluted to specific volumes. Finally, the level of heavy metals were determined against multi-element standard, by inductively coupled plasma optical emission spectroscopy; ICP-OES Optima 3000R (Perkin Elmer, USA). For quality control of honey parameters measured, honey reference material T2818 (FAPAS®, UK) was used. Results were considered acceptable when they were found to be within 2 SD of the mean values given by FAPAS.

Statistical analysis

Data were analyzed using Statistix 9.0 software.[19] Statistical differences between honey samples from different geographic regions were tested by analysis of variance (ANOVA) at $p < 0.05$.

Results and discussion

Statistical analysis indicated that there were no significant differences between honey samples collected from different geographic regions of the West Bank for moisture, relative density, total sugar, reducing sugars, sucrose, HMF, and minerals content. Significant differences were only observed between geographic regions only for pH and protein content.

pH

All honey samples showed acidic pH values, a typical characteristic of floral honey (pH < 4.5).[20] Values of pH ranged between 3.03 and 5.98 with a mean of 3.44 ± 0.11 (Table 1). These values are

Table 1. Quality parameters of Palestinian multi-floral honey from the West Bank.

Parameter	*n*	Mean ± SEM	Range
pH	33	3.44 ± 0.11	3.03–5.98
Relative density g/ml*	32	1.424 ± 0.003	1.422–1.43
Moisture (%)	32	16.53 ± 0.00	14.50–19.00
Total sugar content (%)	32	82.00 ± 0.00	79.00–84.00
Reducing sugar content (%)	21	78.86 ± 0.41	68.96–83.61
Sucrose content (%)	21	4.10 ± 0.41	1.13–6.94
Fructose content (%)	21	38.29 ± 0.47	34.24–41.99
Protein content (%)	21	0.33 ± 0.03	0.20–0.49
Ash content (%)	21	0.14 ± 0.02	0.03–0.21
HMF (mg/kg)	21	12.32 ± 1.80	2.10–34.20

*Relative density = 145/(145 − Baume, 43.16) = 1.424 g/mL.

lower than the range (4.3–6.0) that was reported for Nigerian honeys by Adebiyi et al.[21] but comparable to the range of 3.2 and 4.5 reported by White[22] and to the range of 3.5 and 3.7 reported by Atrouse et al.[23] for Jordanian multi-floral honey from semi-arid regions.. Honey from the Southern Semiarid region of the West Bank had significantly higher pH average (4.39 ± 0.24; $p < 0.05$) when compared to Northwest plains (3.31 ± 0.16), Jordan Valley (3.35 ± 0.22), and Central Mountains (3.20 ± 0.16). This is mainly due to the differences of honey content of different acids and minerals.[24] In general, the lower the pH value of honey, the higher its inhibition of microorganisms activities,[25] as most microorganisms grow best at pH values around 7.

Moisture and relative density

Moisture content is an important quality parameter to evaluate honey.[26] High moisture could increase honey fermentation by certain osmotolerant yeasts,[27] thus reduces honey's storage shelf life time.[28] In addition, high moisture content in some honey may accelerate crystallization process.[29] In the present study, moisture content of all samples analyzed were within the range of acceptable international standard (<20%).[30] It ranged between 14.5 and 19.0% with a mean value of 16.5% (Table 1). Therefore, our results are considered in agreement with the Codex Alimentarius Standard for honey[31] and indicate a good storage ability of Palestinian honey samples analyzed. Comparable results were reported by Atrouse et al.[23] and Eleazu et al.[32] (Table 2). A higher range (17.27–19.80%) of moisture content reported by Boussaid et al.[7] for Tunisian honeys. Differences in moisture content of honey depend on harvest season, the degree of maturity reached in the hive, climatic conditions (moisture level), and water content in the original plant nectar.[20,33–35]

Relative density (specific gravity) and moisture content are correlated and are used as a measure of adulteration in honey. As water content in honey increases, relative density decreases. The mean relative density of the Palestinian honey samples analyzed was 1.4264 with a range of 1.4224–1.4303. These values are similar to those reported by Kamal et al.,[24] Nanda et al.,[36] and Ouchemoukh et al.[37]

In general, honey samples collected from the Northern plains contain the highest moisture content average (16.83%) and the lowest relative density (1.4224) while the Southern semi-arid

Table 2. Quality parameters of Palestinian multi-floral honey compared to those reported from other countries.

Authors/country	pH	Moisture (%)	Ash (%)	HMF (mg/kg)	Reducing sugars (%)
Atrouse et al.[23]/Jordan	3.5–3.7	13.3–18.5			
Aloisi[45]/Argentina	3.2–5.7	5.4–18.4	0.00–0.54	0.00–14.7	
Kamal et al.[24]/Pakistan	3.3–5.8	17.1–17.9	0.05–0.77	16.4–42.9	66.0–79.2
Eleazu et al.[32]/Nigeria	4.3–4.8	15.7–19.3	0.30–0.86		49.1–76.3
Yilmaz and Yavuz[44]/Turkey	3.8–4.5	14.4–18.6	0.06–0.41	0.0–20.4	64.1–76.7
Ochemoukh et al.[37]/Algeria	3.5–4.4	14.6–19.0	0.06–0.54		67.8–80.3
Boussiad et al.[7]/Tunisia	3.3–4.7	14.3–18.6	0.06–0.21	1.48–34.1	63.2–73.2
Present study/Palestine	3.0–6.0	14.5–19.0	0.03–0.21	2.1–34.2	69.0–83.6

region showed the least water content (15.62%) and the highest relative density (1.4303). This is in a good correlation with the climatic conditions of the region where humidity decreases from the north to the south.

Total sugars

The total sugar content in honey samples analyzed averaged 82.0% with a range of 79.0–84.0% (Table 1). Kamal et al.[24] reported a lower range of 69.46–81.63% for different types of mono-floral honey from Pakistan with the Ziziphus honey representing the lowest value while citrus honey represented the highest value in the range. Conti et al.[2] reported the total sugar content of multi-floral honey from Italy as 80.96% (range 77.6–83.8) and in Acacia honey as 81.51% (range 80.2–82.9%). Khalil et al.[38] reported the mean total sugar content in five unifloral honey brands from Bagladesh to be between 77.7 and 80.3%. Generally, total sugar content in Palestinian honey analyzed was either comparable or slightly higher than those reported by other studies.

Reducing sugars

The average content of reducing sugars in Palestinian honey was found to be 78.9% with a range of 68.9–83.6% (Table 1). All values were above the 65% recommended by Codex Alimenarious Commission.[30] A major sugar in honey, fructose, contributed 38.3% of the total reducing sugars in samples analyzed (Table 1). Ouchemoukh et al.[37] reported a similar range of 73.41–77.83% of reducing sugars from polyfloral honey from Algeria. Lower average of 73.4% for floral honey reported by Przybylowski and Wilcyzynska.[39] Citrus honey was reported to have the highest content of reducing sugars with 79.2%.[24] Abu-Tarboush et al.[10] reported a similar value of 79.4% reducing sugars from citrus honey. Palestine is a Mediterranean country that is known for rich citrus production especially the Northwest plains and the Jordan Valley districts. This explains the mean reducing sugar of 78.9% that is very close to citrus reducing sugar content reported above (79.2 and 79.4%).

Sucrose content

The Codex Alimentarious Standard[31] allows a maximum sucrose content of 5% in honey. In our study, the mean sucrose content was 4.1% with a range of 1.1–6.9% (Table 1). Only two samples out of 21 from the Northern plains were above the maximum allowed value of 5% proposed by the Codex Alimentarious Standards.[30] This may be an indication of sugar feeding to the bees or due to floral differences between geographic regions in the West Bank. Honey from sugar fed bees is usually low in water content and pH and high in sucrose content.[10] Lower values of sucrose content (mean = 1.23%) for floral honey were reported by Przybylowski and Wilcyzynska.[39] Also, Ouchemoukh et al.[37] reported a lower range (0.23–3.41%) of sucrose for Algerian poly-floral honey.

HMF

The average HMF content of Palestinian honey was found to be below the limits set by the Codex Alimentarius Standards[31] of not more than 40 mg/kg. The mean of HMF in honey samples analyzed was 12.3 mg/kg and the range was 2.1–34.2 mg/kg (Table 1). The HMF content is used as an indicator of heat processing and/or long storage time of honey.[27] It is formed by the decomposition of fructose in the presence of acids.[40] According to Codex Alimentarius Commision Standards,[41] honey with HMF more than 40 mg/kg indicates heating and more than 150 mg/kg is an indication of adulteration with invert sugar. In general, the Palestinian honey analyzed can be considered of good quality considering its low level of HMF, suggesting freshness and good practices by beekeepers.[42,43] A lower HMF range (0.0–20.4 mg/kg) has been reported by Yilmaz and Yavuz[44] for Turkish honey. Aloisi[45] reported HMF in Argentinian honey as 0.0–14.7 mg/kg, while Kamal et al.[24] reported a higher HMF range for Pakistani honey (16.44–42.9 mg/kg).

Protein

The range of total protein contents of analyzed honey samples was between 0.20 and 0.49% with a mean of 0.33%. Anklam,[46] reported that, normal honey content of protein is <5 mg/g. Similar range of protein content of 0.1–0.5% was reported by and Jagdish and Joseph.[47] A higher value of 0.64% protein content was reported by Nazarian et al.[48] and a higher total protein range for polyfloral honey of 3.7–6.9 mg/g was reported by Ochemoukh et al.[37] Moreover, a lower range of protein content (0.14–0.16%) from several types of Tunisian honey was reported by Boussaid et al.[7] In the present study, honey samples from the Jordan Valley region were found to have significantly higher protein content (0.47% ± 0.06; $p < 0.05$) when compared to the honey from Central Mountains of the West Bank (0.25% ± 0.05). Although most honey proteins are enzymes added by bees, high protein content of honey could be an indication of high pollen content[48] which indicates natural, good-quality honey.

Ash

The mean ± SEM and the range of mineral content of Palestinian honey samples analyzed are shown in Table 3. The ash content in honey constitutes a quality parameter reflecting its richness in minerals that is determined by the botanical origin.[42,49] The average ash content in this study was 0.14% with a range of 0.034–0.214% (Table 1). These results are below the limit of ash content proposed by the Codex Alimentarius Standards[31,41] of not more than 0.6% for natural honey. A similar range of ash content for Algerian polyfloral honey of 0.09–0.21% was reported by Ouchemoukh et al.[37] A higher range (0.08–0.69%) of ash content for Tunisian honey was reported by Boussaid et al.[7] It can be concluded that the Palestinian honey had lower ash contents than those reported by other studies (Table 2).

Elemental composition

In Palestinian honey samples analyzed, the most abundant minerals were K, Na, Ca, P, and Mg with the following mean concentrations 183.86, 104.67, 90.99, 63.75, and 22.74 mg/kg, respectively (Table 3). Adebiyi et al.[21] reported higher concentrations of K and Ca in Nigerian honey samples with K and Ca elements ranked the first and second concentrations among elements investigated. Also, Boussaid et al.[7] reported much higher concentrations for K, Na, and Ca in Tunisian honeys than Palestinian ones. Honey was much less rich in other minerals, namely Fe, Li, B, Mn, and Ba with mean concentrations ranging between 5.21 mg/kg (Fe) and 0.24 mg/kg (Ba). Heavy metals (Cd and Pb) were below detection limits in all honey samples analyzed. Other trace metals were found to follow the following order: Zn > Cu > Cr > Ni (Table 3). Their mean concentrations ranged between 4.13 mg/kg (Zn) and 0.08 mg/kg (Ni). Concentrations of these metals in honey samples from the West Bank seemed to be less than those reported in honey

Table 3. Means (±SEM; $n = 21$) of mineral content(mg/kg) in Palestinian multi-floral honey.

Metals	Mean ± SEM	Range
K	183.86 ± 31.10	42.80–585.00
Na	104.67 ± 14.55	41.80–306.30
Ca	90.99 ± 5.44	44.50–150.70
P	63.75 ± 4.89	39.80–118.10
Mg	22.74 ± 1.91	12.30–46.70
Fe	5.21 ± 0.49	2.00–10.80
Li	3.37 ± 0.54	0.39–8.60
B	2.94 ± 0.45	0.23–6.20
Mn	0.33 ± 0.06	0.11–0.99
Ba	0.24 ± 0.02	0.14–0.60
Zn	4.13 ± 1.23	1.00–19.90
Cu	0.45 ± 0.11	0.00–1.52
Cr	0.17 ± 0.05	0.00–0.74
Ni	0.08 ± 0.03	0.00–0.51

from other countries.[5,21,23,36] However, concentrations of some of these metals in our study were found to be higher than those reported by some authors.[6,44] Mineral results indicated that Palestinian honey is rich in nutritive elements that are important for human health and clean of toxic metals, like Cd and Pb. This indicates safe and high quality honey. Moreover, low levels of heavy metals in honey indicates clean environment.

Conclusions

The results of this study indicated that Palestinian honey physico-chemical characteristics were in accordance with international standards. Honey samples from four geographic regions in the West Bank did not show any significant difference in quality parameters except for pH and protein content. Results indicated honey purity, freshness, and good storage practices by beekeepers. No signs of honey adulteration were observed in any of the samples analyzed. The absence of pollutant heavy metals (Cd and Pb) in all honey samples indicated clean environment while, the richness in other essential metals indicated the high nutritional value of Palestinian multi-floral honey.

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