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Mineral contents and physicochemical properties of natural honey produced in Al-Qassim region, Saudi Arabia

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Abstract

The characterization of natural honeys produced in different areas of Al-Qassim region, Saudi Arabia, was carried out on the basis of either their physicochemical properties or metal contents. The ranges of physicochemical properties were: moisture 14.45-15.95%, total sugars 58.98-80.60%, pH 3.88-4.58, total acidity 10.90-21.84 meq kg^{-1}, ash content 0.139-0.398% and colour light amber-yellowish. According to these values, honey samples met all national and international specifications. Heavy metal analysis was carried out by using atomic absorption spectrometry to detect pollution in the provinces where the honey samples were collected. Mg was the most abundant element in honey samples followed by Fe, Zn, Mn, Cu, Pb and Cd. All these metals were found in non-significant values and are in safety baseline levels for human consumption.

Key words: Natural honeys, metal content, physicochemical properties, atomic absorption, Al-Qassim.

Introduction

Honey is the natural sweet substance produced by honey bees from the nectar of plants, which bees collect, transform by combining with specific substances of either own, deposit, hydrate, store and leave in honeycomb to ripen and mature [1]. Honey possesses valuable nourishing, healing and prophylactic properties [2]. Honey is perhaps one of the most complex foodstuffs produced by nature and certainly the only sweeting agent that can be used by humans without any processing [3]. These properties result from its chemical composition. The various chemical components of honey include sugars which represent the largest portions of honey composition, about 82% [4], protein that includes a number of enzymes (diastase, invertase, glucose oxidase, catalase, etc.) and free amino acids [5]. The composition of honey depends highly on the type of flowers utilized by bees, climatic conditions in which the plants grow and maturation [6, 7]. Many investigators have suggested the use of the physicochemical criteria (water content, sugar content, pH, ash, etc.) analysis for characterization of honey [8-10].

Bee honey can be a good source of major and trace elements needed by humans, it contains metals up to 0.17%. Metals such as Cr, Co, Cu, Fe, Mn and Zn are essential for humans, and they may play an important role in a number of biochemical processes [11, 12]. Some of them are present at the trace level, being toxic if they exceed safety levels [13]. Honey is the result of a bio-accumulation process useful for the collection of information related to the environment where bees live. Since the forage area of the hive is more than 7 km^2 and the bees come in contact with air, soil and water, the concentration of metals in honey reflects their amount in the whole region. Therefore, honey has been recognized as a biological indicator of environmental pollution [14-18].

As a foodstuff used for healing purposes, honey must be free of any objectionable content and should contain only small amounts of pollutants, such as heavy metals. In Saudi Arabia, especially Al-Qassim region, central of Saudi Arabia region, the consumption of honey as food has increased considerably in recent years due to religion beliefs and utilization for medical purpose. Unfortunately, negligible data are available on composition and the contents of the potentially toxic and non-toxic metals in honeys produced in different provinces located in Al-Qassim region. Therefore, the present study was carried out to provide background information on the levels of some metals in honeys. In addition, total sugars, moisture, ash, pH, total acidity, colour coordinates (L^*, a^*, b^*), chroma (C^*_{ab}) and hue angle (h_{ab}) were analyzed for characterization of honeys.

Material and Methods

Reagents and solutions: All chemicals of analytical-reagent grade. Standards solutions were prepared by adequate dilution of a multi-element standard (1000 mg l^{-1}) obtained from J. B. Baker Inc. (Phillipsburg, NJ, USA). Nitric acid (65%) and hydrochloric acid (36%) were purchased from BDH Chemical Poole, England, while hydrogen peroxide (30%) was from WINLAB, UK. All solutions and dilutions were prepared with deionized water (pH 7.0) of 15 MΩ cm resistivity obtained from a water purification system (PURELAB Option-R, ELGA, UK).

Honey samples: Typical *Apis mellifera* honey samples of multifloral types were collected from the 2004 harvest directly from 5 provinces located in Al-Qassim region (central of Saudi Arabia), namely Buraidah, Unayzah, Al-Malida, Al-Midhnab and

Al-Toaymat. Samples were collected from 5 apiaries for each province, stored in holders and immediately transferred to the laboratory at 4°C until analysis.

Physicochemical properties: The samples of honey were analyzed according to AOAC methods [19] in order to determine moisture, pH, total acidity and ash content. Three replicates were used for each honey sample. Moisture content of honey was determined by the refractometer reading at 20°C and the corresponding moisture % was obtained from the table [19]. The pH was measured by means of a potentiometric pH-meter (Hanna Instruments, Italy) after adding 70 ml of deionized water (pH 7.0) to 10 g of honey and mixed thoroughly. Total acidity was determined by titrimetric method from adding free plus lactone acidities and the results were expressed as meq kg^{-1}. Ash content of honey was determined by calcinations overnight of approximately 5 g of honey sample at 550°C in a furnace to constant weight. Sugars content of honey were determined using an Atago refractometer (Atago Co., Ltd, Tokyo, Japan) provided with a temperature correlation scale to compensate when sample temperature other than 20°C. Sugar contents were expressed as Brix degrees (%).

Colour parameters: Colour parameters were assessed by Lovibond RT100 reflectance tintometer (Enhanced Microspectrometer Technology, Germany) based on the reflectance spectra. The reflectance spectra were measured against white background, directly on the honey at wavelength ranging from 300 to 800 nm, and integrated using Workstation Software package for instrument control, data acquisition, and data analysis were used. The colour parameters were colour coordinates, L^*, a^*, b^*; where L^* represents the perceived lightness, a^* and b^* indicate the change in hue from red to green and from yellow to blue, respectively, chroma, C^*_{ab}, a correlate visual for saturation and hue angle, h_{ab}, is a useful quantity in specifying hue numerically.

Determination of minerals in honey samples: Five ml of 0.10 N of either nitric acid or HCl plus 1 ml of hydrogen peroxide was added to a beaker containing the ash of 5 g of honey sample, stirred and the mixture heated on a hotplate to almost complete dryness. Two ml of the HCl (0.1 N) was added to the contents of the beaker, transferred to volumetric flask and diluted with deionized water to 10 ml. Metals were measured by using atomic absorption spectrometer (AAS, Shimadzu Model AA-6200, Kyoto, Japan), equipped with a hollow cathode lamp, a 10 cm long slot-burner head and air/acetylene flame. The operating conditions adjusted in the spectrometer were carried out according to the standard guidelines of the manufacture. Blank solutions were prepared under identical conditions and the average signal was subtracted from analytical signals of honey samples. Working solutions were prepared directly from the metal standard solution (1000 mg l^{-1}) before determination. The emission wavelength used, the correct coefficient for the calibration straight line, the working linear range and detection limit found for each metal are presented in Table 1. The solution concentration for each elements in the sample C_s (μg ml^{-1}), and the average blank, C_b (μg ml^{-1}) were obtained from the measurement data. The final solution volumes of samples V_s (ml) and blank, V_b (ml) were used. The concentration, C (μg g^{-1}) of each sample in the mass of sample taken, M (g) was calculated as follows: $C\ (\mu g\ g^{-1}) = (C_s V_s - C_b V_b)/M$. Three standard concentrations, 0, 0.25 and 0.5 μg of metal stock solution (1000 μg ml^{-1}), were added to the ash of 5 g of honey sample and then digested as described previously to calculate recoveries.

Results are expressed as mg kg^{-1} of fresh honey. Detection limits are defined as the concentration corresponding to three times the standard deviation of ten blanks. Detection limit values of elements (as μg kg^{-1}) were 0.27, 8, 1.4, 0.37, 0.81, 0.60 and 1 for Mg, Fe, Zn, Cu, Mn, Pb and Cd, respectively. The percentages of recovery ranged from 93 to 103 for the studied elements.

Statistical analysis: Data were calculated as mean ± standard deviation (SD) analyzed using analysis of variance (ANOVA). Probability of 0.05 or less was considered significant. The statistical package of Costat Program [20] was used for all chemometric calculations.

Results and Discussion

Physicochemical properties of honey samples: The chemical composition of the investigated honey samples is shown in Table 2. Honey samples collected from apiaries of Al-Midhnab had the highest moisture content followed by Buraidah, Al-Toaymat,

Table 1. Emission wavelength, slit width, linear working range, sensitivity, detection limit and correlation coefficient of the calibration.

Element	Wavelength (nm)	Slit width (nm)	Linear working range (mg l^{-1})	Sensitivity (μg l^{-1})	Detection limit (μg kg^{-1})	Correlation coefficient (r)
Mg	285.20	0.7	0-50	3.5	0.27	0.9983
Fe	248.33	0.2	0-10	80	8.0	0.9940
Zn	213..90	0.7	0-2	11	1.40	0.9991
Cu	324.70	0.7	0-2	40	0.37	0.9988
Mn	279.50	0.2	0-2	28	0.81	0.9920
Pb	217.00	0.7	0-2	120	0.60	0.9997
Cd	228.80	0.7	0-2	12	1	0.9991

Table 2. Physicochemical properties of honey samples collected from Al-Qassim region, Saudi Arabia.

Province	Moisture (%w/w)	Total sugars (°Brix, %)	pH	Total acidity (meq kg^{-1})	Ash content (%w/w)
Buridah	15.70±1.08c	78.37±7.40c	3.88±0.09a	10.90±1.14a	0.242±0.02a
Unayza	14.80±1.18a	77.95±6.70c	4.58±0.54c	21.84±1.94c	0.398±0.03b
Al-Malida	14.45±0.54a	58.98±2.80a	4.02±0.79ab	12.12±1.19ab	0.139±0.02a
Al-Midhnab	15.95±0.58c	80.60±3.10d	4.25±0.69bc	13.31±2.16ab	0.180±0.11a
Al-Toaymat	15.28±0.73b	62.58±7.60b	4.08±0.56ab	14.62±3.64b	0.165±0.09a
$LSD_{0.05}$	0.41	2.74	0.27	3.32	0.11

Data are expressed as mean ± SD. Means followed by the same coefficient within each column are not significantly different from each other ($p \leq 0.05$).

Unayza and Al-Malida, where the corresponding percentages of moisture were 15.95, 15.70, 15.28, 14.80 and 14.45, respectively, indicating good degree of maturity which are in agreement with Saudi Arabia [21] and current the European Community Directive requirements [1] of <20% moisture. This is partially because of the weather in Al-Qassim region is dry. Similar values were found in Moroccan honeys [22, 23]. Moisture content is highly important for the shelf-life of the honey during storage [24] because a high moisture content causes honey to ferment and spoil. The amount of water in honey is a function of many factors involved in ripening, including weather conditions and the original moisture of the nectar, harvest season, the conditions of storage and the degree of maturity [23, 25].

Honey samples collected from Al-Midhnab had the highest sugar contents followed by Buraidah, Unayza, Al-Toaymat and Al-Malida with mean values of 80.60, 78.37, 77.95, 62.58 and 58.98%, respectively. Honey samples collected from Al-Toaymat and Al-Malida violated the national and international specifications [1, 21, 26], requiring >65% of total sugars. The obtained values of sugar content in the present study are very close to that found in Spanish honeys with total sugars ranged from 54.1 to 83.6% [27], whereas it was from 80 to 83.8% in Italian honeys [28]. The moisture and sugar contents of honey are strictly correlated: anomalous values may be a reliable index of adulterations.

The pH values of all samples collected from all provinces are acidic, with a pH values of 3.88, 4.02, 4.08, 4.25 and 4.58 for Buraidah, Al-Malida, Al-Toaymat, Al-Midhnab and Unayza which within the limits established by the national and international organizations [1, 21, 26] requiring 3.0-5.6 corresponding to that for floral honeys [29]. The mean values of pH samples agree with that reported in many countries by many investigators [22, 27, 30, 31]. pH is a useful index of possible microbial growth. Most bacteria grow in a neutral and mildly alkaline environment, while yeasts and moulds are capable of growth in acidic medium (4.0-4.5) and do not grow well in alkaline media [32].

The higher total acidity values of the tested honey were obtained with samples collected from Unayza (21.84 meq kg^{-1}) followed by Al-Toaymat (14.62 meq kg^{-1}), Al-Midhnab (13.31 meq kg^{-1}), Al-Malida (12.12 meq kg^{-1}) and Buraidah (10.90 meq kg^{-1}). The mean values for total acidity found agreed with those from other studies [33, 34]. None of the tested samples collected from all the tested areas exceeded the limit of 40 meq kg^{-1} established [1, 21, 26] indicating absence of undesirable fermentation. The variation in acidity among different honey types may be attributed to either variation in the contents of organic and inorganic acids due to harvest season [24, 35] or floral types [35]. The pH of the honey is not directly related to free acidity because of the buffering action of the various acids and minerals present [6].

The ash contents in all analyzed samples from all tested provinces were below the limit allowed of 0.6% [1, 21, 26] indicating the cleaning of honey samples and possibly of adulteration with molasses [36]. The highest value of ash content was recorded for samples collected from Unayza (0.398%), followed by Buraidah (0.242%), Al-Midhnab (0.180%), Al-Toaymat (0.165%) and Al-Malida (0.139%). Honey normally had a low ash content and it depends on the materials collected by the bees foraging on the flora [6].

Colour parameters: Table 3 shows the colour data for the different parameters. The higher values of X and Y were recorded for honey samples collected from Al-Malida (9.77, 8.71 units) followed by Al-Toaymat (5.16, 4.61 units), Buraidah (3.42, 3.04 units), Al-Midhnab (3.35, 2.960 units) and Unayza (3.23, 2.88 units). Honeys with low X and Y values showed much less vivid colours. Samples collected from Al-Malida had lightness (L^*) value of 32.81, Al-Toaymat of 24.66, Buraidah of 19.33, Al-Midhnab of 16.98 and Unayza of 16.43 units. Samples having lightness (L^*) values between 6.72 and 40 units are amber in colour, while those having higher L^* values were dark amber in colour [22]. Honey samples showed high values for lightness, typical of yellowish honeys. The colour of honey varies from nearly colourless to dark brown [26].

The chroma (C^*_{ab}) represents the amount of colour and is measured according to the coordinates origin (point of illuminate). The corresponding values of C^*_{ab} were 4.54, 4.44, 3.79, 3.06 and 2.13 units for honey samples collected from Al-Malida, Al-Midhnab, Buraidah, Al-Toaymat and Unayza, respectively. The colour parameters lightness (L^*) and chroma (C^*ab) showed relatively low values in Moroccan honeys [22]. The values of colour variables confirm that the colour of honeys collected from Al-Qassim region ranged from light amber to yellowish in colour. The values of colour variables lightness and chroma found in the present study are close to that reported in Moroccan honeys [22].

Honeys collected from Al-Toaymat showed the highest value for the colour coordinate a^* (15.11 units), while Buraidah showed the lowest value (0.84 units). On the other hand, honeys collected from Buraidah showed the highest value of b^* (3.56 units), while the lowest value was recorded for Unayza (-0.74 units). Moreover, the highest h^*_{ab} value was recorded for honey collected from Unayza (253.39 units), followed by Al-Malida (119.26 units), Al-Midhnab (114.02 units), Al-Toaymat (109.25 units) and Buraidah (84.08 units). Low h^*_{ab} values corresponding very low chroma values, ranged from very dull reddish-brown to more yellow colour [22]. The values of colour variables lightness and chroma found in the present study are very high compared to that in Moroccan honeys [22]. The darker the honey, the greater its antioxidants properties [37]. The difference in colour was because of the botanical origin and the amount of suspended particulates such as pollen [16] which reflect the content of pigments with antioxidant properties [37].

Table 3. Distribution of chromatic variables in Al-Qassim honey samples.

Province	X	Y	Z	L*	a*	b*	C^*_{ab}	h_{ab}
Buridah	3.42±0.20a	3.04±0.18a	0.92±0.07a	19.33±0.69ab	0.84±0.14a	3.56±0.16c	3.79±0.17bd	84.08±2.45a
Unayza	3.23±0.37a	2.88±0.33a	1.00±0.10a	16.43±0.11a	6.71±0.70c	-0.74±0.22a	2.13±0.14a	253.39±9.03d
Al-Malida	9.77±0.73b	8.71±0.65b	2.59±0.16b	32.81±1.33c	7.33±1.30c	3.51±0.52c	4.54±0.41d	119.26±9.34c
Al-Midhnab	3.35±0.35a	2.96±0.32a	0.91±0.10a	16.98±1.14a	3.05±0.28b	2.05±0.42bc	4.44±0.12bc	114.02±9.30bc
Al-Toaymat	5.16±0.23a	4.61±0.21a	1.61±0.08a	24.66±0.73b	15.11±1.59d	0.71±0.33ab	3.06±0.11abc	109.25±9.93b
$LSD_{0.05}$	2.94	2.63	0.75	7.33	2.21	2.37	1.46	6.18

X, Y and X tristimulus values; a^*, b^*, colour coordinates; L^*, lightness; C^*_{ab}, chroma and h_{ab} hue angle. Data are expressed as mean ± SD. Means followed by the same coefficient within each column are not significantly different from each other ($p \leq 0.05$).

Table 4. Levels (mg kg^{-1} fresh weight) of some essential elements in honey samples collected from different areas.

Province	Mg	Fe	Zn	Cu	Mn	Pb	Cd
Buridah	18.885±0.97a	0.310±0.038a	0.205±0.022a	0.257±0.023a	0.188±0.013a	0.038±0.002a	0.002±0.001a
Unayza	20.949±0.95bc	2.062±0.48ab	0.298±0.041a	0.206±0.026a	0.235±0.028ab	0.070±0.004a	0.003±0.001a
Al-Malida	23.212±1.40c	3.195±0.270b	0.746±0.048b	0.341±0.024a	0.373±0.022b	0.055±0.006a	0.037±0.007b
Al-Midhnab	20.676±1.10bc	1.811±0.270ab	0.726±0.064b	0.389±0.030a	0.188±0.014a	0.080±0.007a	0.004±0.001a
Al-Toaymat	18.391±0.78a	2.049±0.357ab	0.691±0.067b	0.244±0.028a	0.221±0.021a	0.062±0.009a	0.003±0.001a
$LSD_{0.05}$	3.34	2.38	0.36	0.19	0.15	0.140	0.03

Data are expressed as mean ± SD. Means followed by the same coefficient within each column are not significantly different from each other (p≤0.05).

Metals analysis: The results of the seven minerals determined in honey samples collected from Al-Qassim region are summarized in Table 4. Three mineral groups can be differentiated: elements that are very abundant, elements in a medium concentration and trace elements. The first group consisted of Mg, which being quantitatively the most important ranged from 18.391 to 23.212 mg kg^{-1}. The second mineral group was composed of Fe, Zn, Cu and Mn, where Fe being the most abundant (0.310-3.195 mg kg$^{-1)}$, followed by Zn (0.205-0.746 mg kg^{-1}), Cu (0.206-0.389 mg kg^{-1}) and Mn (0.188-0.373 mg kg^{-1}). The third mineral group composed of Pb and Cd, the ranges being 0.038- 0.080 and 0.002-0.037 mg kg^{-1}, respectively. Iron was the most abundant element in the Turkish honeys, while Cd was the lowest one [38]. On the other hand, the dominant element in Canary Islands honey is K, followed by Rb, Ca, Na, Mg, Li, Fe, Zn, Sr and Cu [39]. The dark honey exhibited twice as much elements as light honey [40]. Fe, Zn and Mn levels in all the tested provinces were lower than those found in either Moroccan [23] or Irish honeys [41]. On the contrary, the levels of Cu, Mg and Mn in the present study were within the values found in Moroccan honeys of River red gum [23]. Zn, Mn and Mg concentrations were strongly dependent on the kind of botanical origin [42].

The highest Mg levels were in honey samples from Al-Malida (23.212 mg kg^{-1}) followed by those collected from Unayza (20.949 mg kg^{-1}), Al-Midhnab (20.676 mg kg^{-1}), Buraidah (18.885 mg kg^{-1}) and Al-Toaymat (18.391 mg kg^{-1}). The present Mg levels in all the analyzed samples do not pose a health risk to consumers.

In case of Fe, the highest content (3.195 mg kg^{-1}) was in honeys from Al-Malida, followed by Unayza (2.062 mg kg^{-1}), Al-Toaymat (2.049 mg kg^{-1}), Al-Midhnab (1.811 mg kg^{-1}) and Buraidah (0.310 mg kg^{-1}). Fe overload as a result of dietary intake is unusual in the normal population [43]. Therefore, the recorded Fe levels do not pose a health risk to consumers because the concentrations in all tested samples were below the guideline value of 15 mg kg^{-1} [26].

The levels of Zn content were 0.746, 0.726, 0.691, 0.298 and 0.205 mg kg^{-1} in the honeys from Al-Malida, Al-Midhnab, Al-Toaymat, Unayza and Buraidah, respectively. All these values were below the guidline value of 5 mg kg^{-1} [26]. Zinc is a nutritionally essential metal but high intake of it results in gastrointestinal distress and diarrhea [44]. Zn and Fe levels in honeys collected from Kateefash, Saudi Arabia, are much higher than our data [45, 46].

Copper values were 0.389, 0.341, 0.257, 0.244 and 0.206 mg kg^{-1} for honeys collected from Al-Midhnab, Al-Malida, Buraidah, Al-Toaymat and Unayza, respectively. The concentrations of Cu in all tested samples were below the guideline value of 5 mg kg^{-1} [26]. Copper is essential for good health but very high intake can cause adverse health problems, such as liver and kidney damage [47].

The maximum Mn levels observed were 0.373 mg kg^{-1} in honeys from Al-Malida, followed by Unayza (0.235 mg kg^{-1}), Al-Toaymat (0.221 mg kg^{-1}) and Buraidah ≅ Al-Midhnab (0.188 mg kg^{-1}). High values of Mn have been reported in the literature [28, 48, 49], which can be attributed either to the production stages of honey or the region from where the honey has been taken [49].

Table 3 also lists the mean Cd findings for all tested provinces. The levels of Cd in honey samples collected from Al-Malida province significantly differed from those in the other provinces. The observed means of Cd were 0.037, 0.004, 0.003, 0.003 and 0.002 mg kg^{-1} for Al-Malida, Al-Midhnab, Al-Toaymat, Unayza and Buraidah, respectively. The contents of Cd detected in all the tested provinces were below the guideline value of 0.05 mg kg^{-1} [26].

The highest Pb contents were recorded in honey samples from Al-Midhnab, followed by Unayza, Al-Malida, Al-Toaymat and Buraidah with mean values of 0.080, 0.070, 0.062, 0.055 and 0.038 mg kg^{-1}, respectively. The concentrations of Pb in all tested samples were below the guideline value of 0.1 mg/kg^{-1} [26]. The Pb content in honey samples collected from Hungary had an average value of 0.052 mg/kg^{-1} which is very close to our data [50]. On the other hand, lead content in Malaysian honey was found to be 0.91 mg kg^{-1} [51]. The present results are in parallel with other investigations that reported dark honeys have a higher metal content than pale ones [7, 42]. Honeys collected from Al-Malida showed high values for lightness (mean 32.81) and also had higher metal content.

In general, the chemical composition of the investigated honey samples collected from Al-Qassim region, Saudi Arabia, generally fit the standards and met all major national and international specifications. The levels of metals in honeys, determined and assessed for its quality comparing with permissible limits stipulated by various agencies and organizations, showed that the present Mg, Fe, Cu, Zn, Mn, Pb and Cd levels are in safety baseline levels for human consumption.

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