Characterization of Saudi Arabian floral honeys by their physicochemical characteristics and heavy metal contents

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Abstract: *The quality of floral honey produced in Saudi Arabia was evaluated. All samples were analyzed for common physicochemical parameters like moisture contents, acidity, ash, pH, conductivity and heavy metal contents. All samples showed proper maturity, considering the low moisture percentage. The total acidity, below 50 meq/kg, in all the samples indicated the absence of undesirable fermentation. Also, the mean pH around 4.0, is usual for floral honeys. The value for ash and conductivity (0.33% and 402µS/cm) were within the European Community Standards. Among heavy metals, Fe and Zn were detected in all samples, with the mean levels of 2.65µg/g and 0.77µg/g. Nickel was detected only in 20% of samples analyzed. The heavy metal contents of Saudi Arabian floral honey were either comparable or lesser than internationally reported values. Therefore, these can be regarded as safe for human consumption.*

Key Words: Floral honey; Heavy Metals; ICP-AES; Saudi Arabia; Physicochemical parameters

Introduction

There is an increasing interest in the verification of foodstuffs, particularly of natural origin like honey. Honey is one of the most complex foodstuffs produced by nature which is consumed by humans without processing. Honey posses valuable nourishing and healing properties. These properties result from its chemical composition. As honey is the result of a bio-accumulative process, it is also useful for collecting information of the environment within the bees forage area. Honeybees (apis mellifera) accretions are related to air, water and soil. They travel from flower to flower; touch branches and leaves, drink water from ponds and aerosol particles scavenge on their hairy bodies. They are continuously exposed to contaminants present in the wide spread area surrounding the apiary for the duration of their foraging activity. The area of foraging activity associated with an apiary extends over a surface of 7sq.km (approx). Owing to this large surface area, honey has been proposed as suitable bioindicator of chemical pollution [1]. The composition of honey varies with the feeding of the bees; it may be naturally from nectar of flower or artificially by feeding bees with sugar or syrup. Bee honey can be a good source of major and trace elements needed by human body. Their presence in human food is very important, but if they exceed safety levels, they can be toxic. According to some Italian workers, honey may be viewed as an environmental marker [2]. They found large amounts of heavy metals in honeys. Contents of Na, K, Ca, Mg, Cu, Fe, Mn and total ash were dtermind in 21 samples of Spanish commercial honeys [3]. Cu, Cd, Mn, Fe and Mg were determined in Turkish honey by AAS [4]. They suggested honey is useful for assessing the presence of environmental contamination. Rashid & Soltan (2004) determined trace elements in different floral honeys from Egypt by using flame AAS[5]. Nozal et al (2004) have carried out classification of Spanish floral honeys depending upon trace elements and physicao-chemical parameters analysis [6]. Similarly, Terrab et al (2004) have characterized Spanish thyme honeys by their physicochemical characteristics and mineral contents [7].

In Saudi Arabia honey is intrinsic to culture. The Quran, Islam's holy book, refers to honey's medicinal and healing properties. Whether used for its medicinal value, as a restorative or simply for sweetening, a Saudi family consumes 1Kg of honey per month, on average. While honey is used daily, consumption is greater during traditional, religious and festive occasions. Saudi statistics show that Saudi Arabia produces almost 90 tons of honey a year; only 2 to 4 percent of what it consumes. Mostly floral honey is produced in Saudi Arabia. The goal of the present study is the physico-chemical characterization of the floral honeys produced at different farms in

Saudi Arabia, by the analysis of 7 physico-chemical parameters (pH, moisture, ash, conductivity, free acidity, lactonic acidity & total acidity) and the 8 trace elements (Cu, Fe, Zn, Cr, Ni, Pb, Mn, Cd).

Experimental

The present study was conducted using 25 floral honey samples from the Eastern Province (KSA), that were collected from beekeepers. All samples were unpasteurised and were taken no more than three months after extraction, stored in air tight jars, transferred to the lab. and kept at 5^{o}C till analyzed.

Determination of trace elements

Accurate multi-element analysis at trace levels is dependent upon prevention of element contamination. All laboratory equipment used for analysis was made of Pyrex, washed with nitric acid, rinsed twice with triply distilled water and dried in a clean environment. All reagents used for sample preparation were of specpure grade (Merck, Darmstadt, Germany). A Thermo IRIS Intrepid II XDL inductively coupled plasma atomic emission spectrometer was used for metal determinations. The operating conditions are given in Table 1. Five mL of nitric acid (0.1M) were added to the resultant ash, and the mixture was stirred on a heating plate until complete dryness. Then, again, 10.0mL of the same acid were added and brought up to 25mL with ultrapure water (Milli-Q, Millipore). This solution was used for instrumental analysis. A recovery test of the analytical procedure was carried out for some of the metals in selected samples by spiking with aliquots of metal standards and then analyzing them as usual. Acceptable recoveries, >96%, were obtained for the analyzed metals.

Determination of physicochemical parameters

Seven physicochemical parameters were analyzed using the Harmonized Methods of the International Honey Commission [8] and Association of Official Analytical Chemists [9]. The ash percentage was determined by sample (≈10g) calcinations at 500^{o}C in an electric furnace until a constant weight was achieved. Moisture levels were determined by noting the refractive index at 20^{o}C by an Abbe Refractometer, and then using the Wedmore table to convert the measurement to percent moisture. pH was measured with pH meter (Horiba), from a solution containing 10.0 g of honey sample in 75.0mL of ultrapure water. Electrical conductivity of the honey

solution (20%) in ultrapure water was measured at 20ºC by a conductivity meter (Horiba) and the results are expressed as µS/cm. The free and lactonic acidities were determined by titrimetric procedure. Free acidity was determined by titrating to pH 8.5 by adding 0.05M NaOH. Lactonic acidity was determined by immediately adding a volume of 10.0mL 0.05M NaOH and back titrated with 0.05M HCl to pH 8.3. Total acidity was obtained by adding the two.

Results and Discussion

The concentration of eight elements determined in the floral honey samples is shown in Table 2. The most abundant element in the honeys analyzed was Fe (X=2.65µg-g^{-1}); whereas Cd showed minimum levels (X=0.05 µg-g-1). From the previous studies it was shown that industrial emissions, automobile exhausts, mining, etc. may cause metallic contamination in honey [10,11,12]. In the study of Morse and Lisk [10], 16 elements were determined in the honey from the US, Mexico, El Solvador and China. They reported rather high levels of trace elements in honey. For example, the levels of Cd 0.102-0.267, Fe 5.8-183, and Ni 0.304-1.25 µg-g-1. The source of these metals was considered as steel or galvanized containers used in processing, shipping or storage. Crane [13] determined the concentrations of Fe, Cu, Mn and other minerals in floral honeys; the mean values were Fe 2.4, Cu 0.29 and Mn 0.30 µg-g-1. The values obtained in the present work (X=2.65µg-g-1) were lower than those reported earlier [5,10] but comparable to the ones reported for Spanish honeys [6] (Table 4). Feryal and Ozlem [4] have reported quite high cadmium concentrations (0.31-0.34 µg-g-1), compared to our values at (0.05-0.1 µg-g-1). Similarly, they have reported Cu as 0.0-0.09 µg-g-1 as against our values at 0.06-0.17 µg-g-1). However, Nozal et al [6] have reported 0.2 µg-g-1 Cu in the Spanish honey whereas Rashed [5] have reported 1.70 µg-g-1 Cu in the Egyptian floral honeys. Lead concentrations found in the present study (0.06 µg-g-1) compared well with the values reported by Przybytowski in the Polish honeys (0.070 µg-g-1) [1] and Spanish honeys (0.03 µg-g-1) [6]. However, lead was detected in 28% honey samples only. Nickel was detected in 20% samples only with the average levels of 0.06 µg-g-1. Very low Ni levels in honey have been reported in literature (Table 4). Zinc was detected in all the samples with an average of 0.77 µg-g-1. Przybytowski have obtained significantly higher levels of Zn in Polish honey samples (22.3 µg-g-1), exceeding the Polish standard limit of 15 µg-g-1[1]. Tuzen

et al [19,20] have reported concentration of Pb and Cd in Turkish honey ranging from 8.4-105.8μg/kg and 0.9-17.0μg/kg, respectively.

Table 3 shows the data pertaining to the analysis different physicochemical parameters of honey samples. Moisture content, a parameter that is related to climate, season and degree of honey maturity [14] was found within 14-20.5%. Only one sample exceeded the permitted limit of 20%, set by European Community Directive [15]. The values found show mature honeys, may be due to use of modern hives by beekeepers and giving proper time of extraction. Ash content, the parameter used for the determination of botanical origin (floral, mixed or honeydew) showed values between 0.13 to 0.56%. None of the samples surpassed the permitted value (0.6%) for floral honey [15]. The mean value was 0.33%, similar to the one reported by Terrab et al [7] and Nozal et al [6] but quite higher than the value found by Sanz et al (X=0.18%) [16].

pH value is of great importance during the extraction and storage of honey. It influences the texture, stability and shelf life of honey. Our samples showed the range 3.5-4.8 with a mean value of 4.0, which is slightly lower (4.2) than the one found by Terrab et al in Spanish honey [7] and higher than the one reported by Accorti et al (3.8) in Italian honey [17]. The electrical conductivity of the honey is directly related to the concentration of inorganic salts, organic acids and proteins. This parameter shows great variability according to the floral origin and considered best for differentiating between honeys of different floral origin [7]. This parameter showed values between 280 to 523μS-cm^{-1} the mean value being 402 μS-cm^{-1}; quite similar to the one reported in Spanish honeys (395 μS-cm^{-1}) but a bit higher reported for the Italian thyme honeys (X=380 μS-cm^{-1}).

Due to the presence of organic acids in equilibrium with their corresponding lactones, or internal esters, and some inorganic ions such as sulphate or phosphate, the values of the free acidity ranged between 18.3-41.7 meq-kg^{-1}. The lactonic acidity is the reserve and is used when the honeys become alkaline, ranged between 4.2 to 9.6 meq-kg^{-1}; while the mean of the total acidity was 34.7 meq-kg^{-1}. None of the samples exceeded the acidity limit established by European community regulations [15]. The results obtained for the acidity compared well to those obtained for Spanish floral honeys [7] but were slightly lower than those reported for Italian honeys (X=43.3 meq-kg^{-1}).

The physicochemical parameters such as moisture contents, acidity and electrical conductivity showed values in agreement with legal limits set by European Union (The Council of European Union, 2002). Among heavy metals Saudi Arabian floral honey showed levels either comparable or lesser than internationally reported values. Therefore, these can be regarded as safe for human consumption.

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Table 1: The operating conditions for ICP-AES instrument

ICP-AES	IRIS Itrepid II XDL
Plasma Conditions	
Rf Frequency	30MHz
Rf Power	1.0kw
Gas Flow Rate	
Carrier Gas	Ar 0.5L/min

Auxiliary Gas	Ar 1.0L/min
Coolant Gas	Ar 20L/min
Sampling Conditions	
Observation Height	18mm above coil
Nebulizer	cross-flow type
Sample uptake rate	1.2mL/min
Spectrometer Conditions	
Polychromator	Paschen-Runge mounting
Focal Length	75cm
Entrance slit	25μm
Exit slit	50 μm
Data Acquisition	
Integration Time	10sec
Repetition	3 times

Table 2: Levels of selected heavy metals (µg-g^{-1}) in floral honey from Saudi Arabia

Cu	Fe	Zn	Cr	Ni	Pb	Mn	Cd
0.08	2.63	0.83	ND	ND	0.06	0.07	ND
0.06	4.12	0.65	ND	ND	ND	0.07	ND
0.13	3.78	1.77	0.12	ND	ND	0.05	ND
0.11	7.89	0.55	0.09	ND	0.05	0.11	0.05
0.07	5.66	0.38	0.07	ND	ND	0.08	ND
0.08	1.63	0.5	ND	0.06	ND	0.13	0.05
ND	2.33	0.47	ND	0.07	ND	0.1	0.08
0.07	0.97	0.63	0.08	ND	ND	ND	ND
0.06	1.35	0.51	0.06	ND	ND	ND	ND
ND	1.6	0.95	ND	ND	ND	0.06	0.1
0.1	2.11	1	ND	ND	0.08	ND	ND
0.09	1.86	0.7	0.05	ND	ND	ND	ND
0.11	0.75	0.68	ND	ND	ND	0.09	0.06
0.06	2.53	2.11	ND	ND	0.06	0.14	0.06
0.17	1.42	0.78	0.07	0.05	ND	ND	0.07
0.09	3.37	1.35	0.05	ND	ND	0.08	ND
0.15	2.89	0.19	0.11	ND	ND	ND	ND
0.16	3.36	0.68	0.08	ND	0.05	0.19	0.06
0.12	4.12	0.33	ND	0.06	0.05	0.06	0.05
0.08	2.78	0.87	0.18	ND	ND	ND	ND
0.13	1.56	1.53	0.05	ND	ND	ND	ND
0.15	1.08	0.48	0.06	ND	0.07	0.05	ND
0.09	2.47	0.71	ND	0.05	ND	0.18	ND
0.12	1.96	0.4	ND	ND	ND	ND	0.05
0.07	2.13	0.29	ND	ND	ND	ND	ND
R 0.06-0.17	0.75-7.89	0.19-2.11	0.05-0.07	0.05-0.07	0.05-0.08	0.05-0.19	0.05-0.10
X 0.10	2.65	0.77	0.06	0.06	0.06	0.10	0.06

ND Not Detected; R Range; X Average

Table 3: Distribution data for common physicochemical parameters for Saudi floral honey

Moisture(%)	Ash(%)	C onductivity (µS/cm)	pH	Free Acidity (meq/kg)	Lactonic Acidity (meq/kg)	Total Acidty (meq/kg)
16.3	0.25	375	4.3	19.3	8.3	27.6
16.8	0.31	480	4.2	31.2	7.2	38.4
16.2	0.19	410	3.6	30.3	5.3	35.6
16.9	0.16	315	3.6	23.7	6.9	30.6
16.5	0.45	300	4.4	27.4	7.1	34.5
17.5	0.51	366	4.5	21.8	6.9	28.7
18.8	0.36	488	4.0	19.7	7.1	26.8
15.8	0.56	510	3.6	29.8	8.3	38.1
16.7	0.39	323	3.5	25.2	9.1	34.3
15.3	0.28	389	3.9	31.3	5.3	36.6
15.0	0.30	362	3.9	18.3	6.1	24.4
14.0	0.26	518	4.0	41.7	4.3	46.0
14.5	0.44	451	4.6	24.5	4.2	28.7
17.8	0.27	497	4.3	31.7	6.3	38.0
20.5	0.30	410	4.8	27.8	5.8	33.6
19.3	0.14	280	4.6	33.5	7.2	40.7
17.2	0.17	379	4.2	29.9	5.4	35.3
16.5	0.30	351	4.1	37.9	9.6	47.5
16.0	0.13	523	3.9	32.3	8.1	40.4
16.9	0.52	341	3.7	26.7	7.5	34.2
15.3	0.31	455	3.5	34.9	6.6	41.5

18.3	0.53	463	3.6	25.7	9.0	30.7
18.7	0.41	353	4.1	23.5	8.5	32.0
17.5	0.44	341	4.2	24.7	6.3	31.0
16.9	0.37	370	4.0	26.3	5.6	31.9
X±SD *16.8±1.5*	*0.33±0.13*	*402±73.1*	*4.0±0.3*	*27.9±5.7*	*6.8±1.5*	*34.7±5.8*

Table 4: International comparison of the present data

	This Work	Turkey [4]	Spain [6]	Poland [1]	Egypt [5]	Italy [18]
Cu	0.06-0.17	0.0-0.09	0.1-0.3	NA		NA
X±SD	0.1±0.03	0.01	0.20		1.7	
Fe	0.75-7.89	0.04-1.21	1.0-3.3	NA		NA
X±SD	2.65±1.59	0.36	1.97		202	
Zn	0.19-2.11	NA	0.3-1.5			NA
X±SD	0.77±0.47		1.07	22.3	7.2	
Cr	0.05-0.18	NA	0.0-0.2	NA	NA	
X±SD	0.08±0.04		0.13			0.094
Ni	0.05-0.07	NA	0.0-0.1	NA		NA
X±SD	0.06±0.01		0.03		1.25	
Pb	0.05-0.08	NA	0.0-0.1			
X±SD	0.07±0.01		0.03	0.07	6.3	0.045
Mn	0.05-0.19	0.0-0.09	0.1-0.4	NA		NA
X±SD	0.1±0.04	0.03	0.30		1.7	
Cd	0.05-0.1	0.31-0.34		0.50		
X±SD	0.06±0.02	0.32			0.50	0.025

X Mean; SD Standard Deviation; NA Not Available