

RESEARCH ARTICLE

Evaluation of physico-chemical properties of honey samples collected in Erbil /Iraq

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ABSTRACT

The present study was conducted to evaluate the physico-chemical properties of honey which were collected in Erbil city and the surrounding area. The total number of samples gathered was 12. The physico-chemical properties were moisture content, pH, ash, electrical conductivity (E.C), hydroxymethyl furfural (HMF), colour, minerals and sugar contents. HPLC method was used to determine the sugar content profile (fructose, glucose and sucrose) while minerals content were assessed by ICP/mass spectrometry. The mean values of fructose, glucose and sucrose were as follows; 38.86, 33.60 and 11.70g/100g. The sucrose level in all samples exceeded the permissible amount as stated by Codex Alimentary commission. The moisture content range was between 11.0% to 16.6% while the pH values were lies in the range 3.88 to 4.15 and HMF content average value were 8.07mg/100g. The furthermost abundant elements in honey samples were K, Ca, Na, Mg, P, S and Fe with mean values of 793.4, 428.7, 202.4, 197.6, 103.1, 73.0 and 19.9 mg/kg, respectively

Keywords: : Honey, quality, physicochemical, properties.

sucrose), minerals, ash, HMF (hydroxymethyl furfural),

INTRODUCTION

Honey, one of the main products of bees, is a natural sweet viscous fluid made from the nectar of plants or from secretions of living parts of plants (Iftikhar F. et al., 2014). It is a concentrated aqueous solution of many compounds, the major components are mono and disaccharides besides that it contains organic and amino acids, minerals, enzymes, vitamins, aromatic substances, waxes, pigments and pollen (Qiu P et al., 1999 and Bogdanov S. et al., 1999). Honey is produced in the worldwide and is very important energy food. It is used for dietary; industrial and medicinal purposes and regarded as an important product in the international market and especially in our local market (Buba F. et al., 2013).

Many researchers (Gürbüz, S. et al., 2020 , El Menyiy N. et al., 2020 and Carina F. et al., 2020) have described physical and chemical properties of diverse types of honeys. Effects of major chemical constituents on the quality of honey also have been studied. Nowadays, there is a worldwide increasing request by consumers to natural foods and diets demanded to improve human health. Honey has a significance role in this field because it contains oligosaccharides beside a wide range of other important nutrients (Vijayakumar K. et al., 2020).

Honey is commonly assessed by a physicochemical analysis of its ingredients. The physicochemical properties which studied by many researchers were sugar contents (fructose, glucose and

proteins, vitamins, moisture, pH, electrical conductivity (E.C) ...etc. The quality of honey is directly affected by its physical and chemical properties, and in turn it is affected by other factors, like the kind of flowers that bees feeding on, geographical region and climate (da Silva P. M. et al., 2020). This study was conducted to check the quality of honey samples collected in local market as well as for contrast with Worldwide Honey Values to get a beneficial fact for the honey consumers.

2. METHODS AND MATERIALS

All chemicals used were of analytical reagent and distilled water used throughout the study.

2.1. Samples Collection

Twelve samples were collected randomly from Erbil city market and also from beekeepers during 2019 honey harvesting season. Table 2.1 elucidates the location of each sample.

Table 2.1: Geographical location of collected honey samples

Sample No	Location name
S1	Rawnduz
S2	Choman
S3	Samilan
S4	Shaqlaw
S5	Rayat

S6	Qushtapa
S7	Balisan
S8	Horney
S9	Sidakan
S10	Karokh
S11	Haji-omeran
S12	Khoshkan

2.2. Instruments

A T80+ UV-Visible spectrophotometer, Sartorius pH-meter, Abbe refractometer (00431) and Jenway 4200 conductometer were used in the present work.

2.3. Determination of Moisture Content

Moisture content of honey samples was determined by using Abbe refractometer method. The refractive index was read at 20°C and the corresponding percentage of moisture from AOAC standard was obtained (W. Horwitz, 2010).

2.4. pH

A ten-gram sample of honey was dissolved in 75 milliliters of distilled water. A magnetic stirrer was used to mix the solution. In the solution, the pH electrode was immersed. The pH of the honey solution was then measured (Bogdanov S. et al., 1997).

2.5. HMF

The concentration of 5-(hydroxymethyl)-furan-2-carbaldehyde was determined using the following procedure (HMF). HMF levels are typically measured in milligrams per kilogram (mg/kg). To evaluate the HMF content, a 5.0g honey sample was carefully weighed into a beaker, and then 25.0 ml of d.w. was added and well mixed until the honey samples were completely dissolved. After that, the combined solution was poured into a 50ml volumetric flask. In the volumetric flask, 0.5 ml of Carrez Solution I (15.0g K₄Fe(CN)₆·3H₂O/100ml d.w) was added and thoroughly mixed, followed by 0.5 ml of Carrez Solution II (20.0g Zn(CH₃COO)₂·2H₂O/100ml d.w). Distilled water was used to dilute the solution to the desired concentration. It's possible that a drop of ethanol will be required to keep the foam from forming during the mixing process. After that, a filter paper was used to filter the mixture. The first ten milliliters of filtered fluid were discarded, but the remaining solution was collected. 1.0 mL of the filtrated solution was pipetted into two separate test tubes. In the test tube containing the honey solution, 1.0 ml of distilled water was added for the sample solution. The reference solution was thoroughly mixed, then 1.0 mL of 0.2 percent sodium bisulphate (0.2g NaHSO₃/100mL d.w.) was added and thoroughly mixed. Using a 1cm quartz cell, the absorbance of the sample solution versus the reference solution at 284nm and 336nm was measured within an hour. If the sample absorbance at 284nm was greater than 0.6, it should be diluted with distilled water and the reference with 0.2 percent bisulphite to provide a sample absorbance low enough for accurate results. This relationship was used to determine the HMT (Sereia M. J. et al., 2017).

$$\text{The HMF content (mg/kg)} = \frac{(A_{284} - A_{336}) \times 149.7 \times 5}{\text{weight of sample}} \quad (1)$$

A₂₈₄=absorbance of sample against reference

A₃₃₆=absorbance of sample against reference

2.6. Electrical conductivity

Twenty grams of anhydrous honey was dissolved in distilled water in a beaker and the solution transferred quantitatively in to a 100 ml volumetric flask. The solution was placed in water bath to adjust temperature at 20 °C. Then conductivity cell was rinsed thoroughly and immersed in the sample solution and the conductance was read after temperature equilibrium has been reached (M. Piazza M. et al., 1991)

2.7. Ash

The ash content of honey samples was determined depending on the linear relationship between the ash content and the electrical conductivity employing the following relation:

$$C = 0.14 + 1.74 A \quad (2)$$

where C is the electrical conductivity in milliSiemens per cm and A the ash content in g/100 g of honey (Bogdanov S. et al., 1999).

2.8. Colour

For the determination of the nature of colour, a uv-visible spectrophotometer was used. The spectrophotometer set at zero using glycerin as a blank on 560nm and employing 1cm matched quartz cuvette. The readings were taken directly from the instrument for the honeys. The honeys were categorized depending on absorbance using next [Table 2.2](#) (Sereia M. J. et al., 2017).

Table 2.2: Honey colour corresponds to absorbance's

Color	Color range
Water white	0.030 or less
Extra white	More than 0.030–0.060
White	More than 0.060–0.120
Extra light amber	More than 0.120–0.188
Light amber	More than 0.188–0.440
Amber	More than 0.440–0.945
Dark amber	More than 0.945

2.9. Determination of Sugar Content

The high-performance liquid chromatography instrument (Knauer Azura HPLC system) was used for fructose, glucose and sucrose analysis employing refractive index detector (Victorita B. et al., 2008.)

2.10. Elemental analysis

Elemental analysis was carried out using inductively coupled plasma (ICP)/mass spectrometry. The Agilent 7500 (USA) instrument was used (Aljohar H. I. et al., 2018).

2.11. Statistical Analysis

The significant differences were obtained by a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison using Graph Pad Prism V9.0 (California corporation) while Correlations were carried out by using the Pearson's correlation coefficient (r) in bivariate linear correlations using Excel Microsoft program (El Menyiy N. et al., 2020).

3. RESULTS AND DISCUSSION

3.1. Sugar composition

The major constituents of honey are carbohydrates. Table 3. 1 shows the sugar contents of honey samples. The total sugar contents ranged between 80.71-88.43g/100g and the fructose to glucose ratio was between 0.958 to 1.250. The low value of fructose to glucose ratio (less than 1.3) increases possibility of

crystallization of honey (A. Agus and N. Umami 2019). Fructose is the main content in honey samples among other sugars (average=38.86g/100g) then glucose with average=33.69 g/100g after that the sucrose comes (average=11.7 g/100g). The sucrose concentration in all samples were high and it exceeded the Codex Alimentary level (5.0g/100g), however, the same organization has clarified another percentage of sucrose in honey which is 15% for some distinct types of honey, this high concentration of sucrose may be due to feeding the bees with

sucrose syrup by beekeeper (W. H. Organization, 2001). The results show significant difference ($p > .05$) among all types of honeys relating to the glucose content while no significant differences were observed concerning sucrose and fructose contents. Honey usually contains much more sugars than fructose, glucose and sucrose including simple sugars, disaccharides and oligosaccharides it may reaches 14 types of sugars (Ahmed M. et al., 2014).

Table 3.1: Sugar contents of honey samples

Sample No	Sucrose (g/100 g)	Glucose (g/100 g)	Fructose (g/100 g)	Total sugar (g/100 g)	Fructose/Glucose ratio
S1	19.60	28.00	35.02	82.62	1.250
S2	11.62	35.27	33.82	80.71	0.958
S3	8.50	34.89	44.83	88.22	1.284
S4	18.26	29.85	35.56	83.67	1.191
S5	9.06	36.94	40.68	86.68	1.101
S6	8.38	37.4	42.65	88.43	1.140
S7	10.74	34.77	40.7	86.21	1.170
S8	11.29	33.22	38.07	82.58	1.145
S9	11.89	32.08	37.8	81.77	1.178
S10	11.74	34.14	38.7	84.58	1.133
S11	9.59	34.39	36.96	80.94	1.074
S12	9.89	33.43	41.60	84.92	1.244

3.2. pH

The honey samples showed a pH from 3.88 to 4.15, with an average of 4.02 ± 0.085 . The low pH of honey inhibits growth and incubation of microorganisms. The pH value has very important role during extraction and storage of honey as it affects the stability, texture and shelf life of honey. The results of pH in the present study agreed with results obtained by other studies (Iftikhar F. et al., 2014, Aljohar H. I. 2018 and H. S. Salih and S. H. Al-Jaf, 2019) in which it is not less than 3.2 and not more than 4.7. The results indicate no significant difference ($p < .05$) among honey samples pertaining to pH values.

3.3. Moisture content

Good quality honey can't contain more than 18% water. However, honeys of tropical origin contain more water than

this level due to exceed humidity. High water level can cause the process of fermentation of honey and loss of its quality (Posudin Y. 2016). The mean moisture content of collected honey samples was $14.05\% \pm 1.63$ (Table 3.2) and ranged between 11.0% to 16.6%. The results of moisture content were within acceptable range ($\leq 20\%$) according to Codex Alimentarius Commission (W. H. Organization 2001). The moisture content depends on diverse factors such as the environmental temperature, harvesting season and the maturity of the hives (Acquarone C. et al., 2007). The results show significant difference ($p > .05$) among honey samples concerning to moisture values. The chance of fermentation in harvested honey samples will be reduced because the moisture content was less than 18.0% (M. Lees and J.-F. Morin 2018).

3.5. HMF

Hydroxymethyl furfuraldehyde (HMF) is the decomposition

3.4 Color

Varieties within the colors of the honey related to its floral origin, mineral content, storage and product processing, climatic factors during nectar flow and the temperature at which the honey matures in the hive, as well as factors like the proportion of fructose and glucose present (Sereia M. J. et al 2017). The dark color of honey was ascribed to a higher mineral content (ash) than in lighter ones. Appositive relation (0.427) was existing between color intensity and ash content (Table 3.4). The samples color were white, extra light amber, light amber and amber (Table 3.2). The predominant color was amber among other colors.

product of fructose. The amount of HMF in honey is one of the most significant indicators of its freshness. Fresh honey contains

only trace amounts of HMF, but its concentration increases with storage and prolonged heating (T. Gebremariam and G. Brhane 2014). The HMF contents of samples of honey presented in Table 3.2. The results of HMF ranged from 0.149 to 45.059 mg/kg with mean value of 8.070 ± 15.210 mg/kg of honey. The results point to no significant difference ($p < .05$) among honey samples regarding to HMF contents. Among the honey samples which were collected one sample (S6) contains higher than the maximum permissible amount (40 mg/kg honey) according to Codex Alimentarius Commission (W. H. Organization 2001). The high value indicates that this sample (S6) had been heated and/or adulterated with processed sugar.

3.6. Electrical conductivity (E.C)

Conductivity is a good yardstick of the botanical origin of honey. Honeydew honey and blossom honey are distinguished by Electrical conductivity values. Honeydew honey characterized by high Electrical conductivity value more than 800 ($\mu\text{S}/\text{cm}$) while blossom honey has electrical conductivity

less than 800 ($\mu\text{S}/\text{cm}$) (Thrasylvoulou A. et al., 2018). Table 3.2 shows the E.C values of honey samples which were in the range 172-748 $\mu\text{S}/\text{cm}$. The results are within the Codex standard limits (W. H. Organization 2001) and similar with those results which were reported by Salih and Al-Jaf (H. S. Salih and S. H. Al-Jaf, 2019) and show that the harvested samples from blossom honey origin (Karimah U. et al., 2020, de Almeida L. B. et al., 2013 and M. A. Sgariglia M.A. et al., 2010). The results of conductivity measurement indicate very high significant difference ($p > .05$) among collected samples.

3.7. Ash

Honey's mineral content is normally low and is determined by the nectar composition of the plants used in its processing. Ash content shows the richness of honey in mineral content. The higher mineral content, the higher ash value. From Table 3.2, the ash values of honey samples were ranging from 41.37 to 429.06 mg/100g, where the S6 sample showed the highest ash value. No significant difference ($p < .05$) was found between samples concerning ash content.

Table 3.2: Physical and chemical properties of honey samples

Sample No.	Moisture content %	pH	E.C ($\mu\text{S}/\text{cm}$)	ASH mg/100g	HMF (mg/kg)	Color
S1	14.3	4.14	172	98.59	2.395	Light amber
S2	16.6	4.06	279	159.98	0.149	Amber
S3	11.0	3.88	390	223.67	2.245	Light amber
S4	15.6	4.08	381	218.50	2.844	Extra light amber
S5	13.0	3.91	539	309.15	0.149	Light amber
S6	12.4	3.96	748	429.06	45.059	Amber
S7	13.0	4.15	520	298.25	35.508	Amber
S8	12.5	4.05	228	50.57	2.08	Amber
S9	15.4	4.05	225	48.85	1.359	Extra light amber
S10	13.8	3.95	217	44.25	2.749	Light amber
S11	15.7	4.05	212	41.37	0.222	White
S12	15.4	4.00	221	46.55	2.09	Light amber

3.8. Mineral Contents

The flavor and color of honey are influenced by the mineral content: honeys with the highest ash contents are darker (Sgariglia M.A. et al., 2010). The existence and quantity of the following elements was checked by ICP/mass spectrometry in the honey samples: Ag, Al, As, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Hf, Hg, Ho, In, K, La, Li, Lu, Mg, Mn, Mo, Na, Nb, Nd, Ni, P, Pb, S, Sb, Sc, Se, Sm, Sn, Sr, Ta, Tb, Te, Th, Ti, Tl, Tm, U, V, W, Y, Yb and Zn. Most of the elements were not detected by ICP/mass

spectrometry and the detected elements are presented in Table 3.3. The most abundant elements were K, Ca, Na, Mg, P, S and Fe with mean contents of 793.4, 428.7, 202.4, 197.6, 103.1, 73.0 and 19.9 mg/kg, respectively. The amount of minerals which present in the honey ranges from 0.04% to 0.2% according to Alqrni (Alqarni A. S. et al., 2014) where these quantities depend on type of plants which bees feeding on it in turn it depends on geographical region of these plants. The quantity of heavy metals in these honey samples prove that they were not polluted.

Table 3.3: Minerals reading of honey samples

Sample no.	Al	Ba	Bi	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	P	S	Sr	Zn
S1	0.41	ND	ND	173.9	ND	0.5	10.6	161.6	131.1	ND	106.7	0.88	36.1	10.1	0.51	ND
S2	1.7	0.66	ND	311.1	ND	1.9	24.24	626.8	184.7	ND	151.3	1.26	106.7	39.3	0.66	0.66
S3	1.19	ND	ND	304.4	ND	1.4	28.22	641.5	369.8	0.54	279.2	2.21	109.9	325	1.49	0.5
S4	ND	ND	ND	292.8	ND	0.8	11.17	322.3	274.4	ND	280.8	1.17	71.81	56.6	2.39	ND
S5	2.01	2.99	0.6	1128.6	ND	1.3	25.59	1252.8	248.2	2.18	335.6	0.64	146.1	54.7	7.08	1.0
S6	2.73	3.52	ND	1192.9	0.57	2.2	38.27	2124.1	312.7	1.93	399.4	0.81	225.2	55.8	6.6	2.05
S7	1.62	1.71	ND	288.8	ND	1.2	17.95	1588.4	161.1	1.99	152.1	0.67	108.9	72.2	2.14	1.0
S8	1.04	ND	ND	280.1	ND	1.0	16.99	637.6	122.8	ND	109.5	ND	92.8	68.3	0.72	1.81
S9	0.82	ND	ND	248.8	ND	1.1	16.04	385.4	113.8	ND	225.3	ND	73.1	40.3	0.75	0.5
S10	ND	ND	ND	304.1	ND	0.6	18.53	309.5	180.4	ND	120.1	ND	76.4	63.3	1.2	0.8
S11	0.56	ND	ND	283.5	ND	1.1	13.69	326.5	82.6	ND	68.7	ND	59.6	60.6	0.84	ND
S12	ND	1.46	ND	336.1	ND	1.2	17.56	1144.8	190.0	ND	200.4	ND	130.9	30.0	3.41	0.73

Table 3.4: Pearson correlation coefficient between studied parameters

	pH	moisture	E.C	Ash	HMF	C. I	Fructose	glucose	sucrose
pH	1								
moisture	0.478	1							
E.C	-0.300	-0.505	1						
Ash	-0.222	-0.473	0.968	1					
HMF	0.112	-0.395	0.781	0.722	1				
C. I	-0.011	-0.441	0.400	0.427	0.489	1			
fructose	-0.658	-0.753	0.542	0.440	0.417	0.229	1		
glucose	-0.603	-0.363	0.616	0.502	0.401	0.351	0.566	1	
sucrose	0.627	0.383	-0.393	-0.240	-	-	-0.691	-0.909	1
					0.265	0.193			

3.9. Pearson correlation

[Table 3.4](#) shows a Pearson correlation between diverse physical and chemical parameters for the honey samples. Karl Pearson correlation coefficient lies between $-1 \leq r \leq +1$.

A strong positive correlation was found between E.C and each of ash and HMF with correlation coefficient 0.968 and 0.781

respectively. This clarifies that those honeys with high ash content take high E.C values. Fructose and glucose in honey samples were negatively correlated with sucrose content ($r = -0.691$ and -0.909). The colour intensity implies no strong correlation with any physico-chemical properties. A negative correlation was existing between moisture content and all other studied parameters except the pH and sucrose.

4. CONCLUSIONS

This study evaluates the physicochemical properties of twelve honey samples. The results revealed that all honeys fulfill the codex Alimentarius requirements except for sucrose content and HMF for only one sample. The sugar content profile was high for all samples while the moisture content was low which reduce the potential of fermentation by microorganisms. The HMF concentration generally was low which confirm freshness and young age of the samples. The samples, to high extent, were free from heavy metals which indicates its clearance from contamination.

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