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Characterization of natural bee honey in different areas of the Sudan

Salah Elnaiem Mohamed, Samah Osman Mohamed
University of Khartoum Faculty of Science Chemistry Department

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****Corresponding Author***

Salah Elnaiem Mohamed

Abstract

Characterization investigation of five natural bee honey samples produced in different areas of Sudan, were carried out based on their physicochemical properties: pH, refractive index,moisture content, viscosity and free acidity ; in addition to sugars ,metals,ash and ascorbic acid contents. These included one sample from Darfur states, Kurdufan states, South Sudan, and another two samples from the Blue Nile State (amber and white honey). Sugar analysis was carried out with High Performance Liquid Chromatography using a refractive index detector (HPLC-RI). Metals analysis carried out by using Flame- Atomic Absorption Spectrometry (F-AAS). Ascorbic acid analysis was carried out using Ultraviolet-Visible spectrometry (UV-Vis). The results were showed significance difference between the different honey samples.

INTRODUCTION

Honey is the natural sweet substance, produced by honeybees from the nectar of plants or from secretions of living parts of plants, or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature[1].

Bees make honey from the nectar that they collect from flowers, other plant saps and honeydew are used to a minor extent. The colour, aroma and consistency of honey all depend upon which flowers the bees have been foraging. Forager honeybees are always female worker bees. The queen bee and drone bees never forage for food[2] The composition and flavour of honey varies, depending mainly on the source of the nectar(s) from which it originates and to a lesser extent on certain external factors - climatic conditions and beekeeping practices in removing and extracting honey [2].

Honey is a useful source of high-carbohydrate food, and usually contains a rich diversity of minor constituents (minerals, proteins, vitamins enzymes and volatile substance which responsible for the characteristic flavor and others), adding nutritional variety to human diets[3].

In many countries, Honey does have medicinal properties that are acknowledged increasingly by modern medicine[4]. Honey is of eaten as an energy food. It is simple sugar directly absorbed into bloodstream without digestion .hone mixes well as sweeteners into hot and cold drinks .It is goes with nearly all foods. From salad dressing to vegetable and meat glazes to Casserole dishes. It is especially good in desserts and baked goods the moisture absorbing quality of honey help breads, Cakes, cookies and candies stay fresh longer[5].

Many scientists have published the physical properties and chemical composition of honey from different sources. Refractive index of honey is primary interest in this property of honey is to provide a rapid, accurate, and simple measure of moisture content[6].

Another physical characteristic of practical importance is density. Honey density, expressed as specific gravity, is greater than water density by about 50 %, and it depends on the water content of the honey [7]. Because of the variation in density, it is sometimes possible to observe distinct stratification of honey in large storage tanks. Color

in liquid honey varies from clear and colorless (like water) to dark amber or black. The various honey colors are all nuances of yellow amber. Honey has also the property to rotate the plane of polarization of polarized light. This property is due to the individual sugars. As a sugar solution, honey has the property of rotating the plane of polarized light. Some sugars (e.g. fructose) exhibit a negative optical rotation, while others (e.g. glucose) a positive one. The overall optical rotation depends on the concentration of the various sugars in honey. Honey is a viscous liquid.The honey viscosity depends on the honey water content and on the temperature [8][9].

Honey pH is affected by the conditions during extraction and storage, which also influences texture, stability and shelf life. The pH of honey found ranged between 3.42 and 6.1 with an average value of 3.91.The acidity of honey can vary widely, the limit quoted in the proposed Codex regulation is not more than 40 Meq acid/ kg honey as determined by direct titration [14].The water content is a quality parameter, important above all for honey shelf life, because of its influence on fermentation. There is a relation between honey water content and the yeast count. Methods for determining the moisture content of honey have been studied for many years such as chemical (Karl Fischer) and several physical methods, including evaporation, distillation, refractometry, density, and viscosity[11].

Honey is primarily a carbohydrate product; sugars are the main constituents of honey, comprising about 95 % of honey dry weight. Main sugars are the monosaccharides hexoses fructose and glucose, which are products of the hydrolysis of the disaccharide sucrose. Besides, about 25 different sugars have been detected. The sugar composition can be determined by different chromatographic methods. The first detailed report on the use of paper chromatography to investigate the identity of the sugars in honey appeared early in 1952. HPLC being the most widely used one[12].

Many authors all over the world have determined the elemental content of honey and a variety of methods has been used. The most popular methods used to determine mineral content of honey are spectroscopic techniques, such as F-AAS, ET-AAS (Electro thermal Atomic Absorption Spectrometry).

Techniques such as ICP-OES, ICP-MS enabled the determination of heavy metals and trace elements in honey owing to their wide range linearity, superior sensitivity and high efficiency [12]. The levels of K, Zn, P, B, Mn, Mg, Cu, Ca, Ba, Sr and Na were determined in Spanish honey. The TXRF and infrared spectrophotometer techniques were used for the determination of mineral content Nigerian honey[13][14]. Thus, the goal of the present work is the physicochemical characterizations of the honeybee produced from different regions in Sudan, by the analysis of seven common physicochemical parameters (refractive index, pH, free acidity, ash, water content, sugar content (fructose, glucose, and, sucrose), and the principal mineral elements (K, Na, Ca, Mg, Fe, Cu, Mn, Co and Zn). The objective of this study was therefore to evaluate the physical properties and chemical properties of honey collected from different regions of the Sudan.

Experimental

Materials and methods:

Sampling:

Five samples of natural bee honey were collected from four geographically different areas.one sample from Darfour states, and the second from Kurdofan states, the third from South Sudan state, and two samples from Blue Nile state (amber and white honey).

Physicochemical analysis:

Six physical parameters, PH, moisture content, refractive index, viscosity, free acidity, and ash content were determined for each sample. Results are shown in table 1.

PH:

PH value was determined according to 350 PH meter -JENWAY. 10g of honey sample were weighed and diluted in 75 cm^3 of distilled water. The PH electrode was immersed in the honey solution and the PH value was recorded.

Refractive index: (R. I)

Refractive index of the five honey samples were determined by KRUSSOptrnic Germany refractometer at constant temperature (26°C).

Moisture content:

The moisture content was determined by calculation and expressed as percentage.

Viscosity:

The viscosity of the five honey Samples were measured at 26^oC using capillary viscometer-schott GERATE.

Free acidity:

10.0 g of honey sample were weighed and diluted in 75 cm^3 of distilled water. Sample was titrated against (0.2M) sodium hydroxide solution using phenolphthalein indicator, the end point was determind by pink color that persisted for seconds. The results expressed as mg/kg honey.

Ash Content:

The ash content was determined using muffle furnace. 5.0 g of honey sample weighed in dry crucible and ignited at 550^{o}C for 2 hours. The ash was determined by calculation and expressed as:

$$Wa = \frac{(m_1 - m_2) \times 100}{m_0}$$

Wa = ash content (g/100g), m_0 = weight of honey taken

m_1 = weight of dish + ash m_2 = weight of dish

Sugar content:

The sugar content of honey samples was determined according to the external standard procedure. The result are shown in table (2).

Instrument:

HPLC: shimadzo- japan

Column: normal- phase (shim-pack)CLC-NH_2 (4.6mm i.dx 15 cm ,5 µm particle size, SHIMADZU,KYOYO,JAPAN) equipped with a guard column of the same material.

Mobile phase: Acetonitrile (75%):Deionized water (25%) (V/ V)

Sample preparation:

2.5 g of honey sample were weighed into a beaker and dissolved in 50 cm^3 water then shaked. The solution was filtered through membrane filter 0.45 µm.20 µl of extract was injected in HPLC-RI detector.

Flow rate: 1.00 ml /min.

Mineral content:

5.0 g of honey sample were weighed in dry crucible; and placed in muffle furnace at 550°C for two hours. The contents were cooled and transferred to 250 cm^3 beaker and, 10 cm^3 of 5M HCl and 1 cm^3 of concentrated HNO_3 were added. The beaker was placed in water bath to boil for 15 mins. 50 cm^3 of distilled water was added and the content was filtered through filter paper and the volume was made to 100 cm^3 with distilled water. The Cu, Fe, Mg, Zn, were determined by F-AAS.

The Na^+, K^+, Ca^{+2}, were determined by flame photometer 410. The results are shown in table (3).

Ascorbic Acid content:

Instruments and Apparatus:

Spectrophotometric measurements were made with a double beam uv 1800 ultraviolet-visible spectrophotometer provided with matched 1.0cm quartz cell (SHIMADZU JAPAN).

Jenway instrument HI 350 pH meter.

Electronic balance type AY220.

Preparation of sample:

1.0 g of five different samples were transferred into 100 cm^3 volumetric flask and diluted to the mark with sodium oxalate solution. The absorbencies of standard solutions and sample were measured at 226nm against the sodium oxalate solution as blank.

Results and discussion

Table (1): pH-values of different honey samples

Physical character	Darfur	kordofan	South Sudan	Blue Nile	white honey
PH	4.61	4.43	4.24	4.76	3.98
RI	1.4885	1.4935	1.4875	1.4885	1.4865
Moisture content (g/100g) (%)	19.20	17.20	20.40	19.60	20.00
Viscosity (poise)	50.42	66.45	12.33	45.14	13.63
Free acidity (mg/kg)	38	43	40	39	146
Ash content (g/100g)	0.220	0.287	0.904	0.552	0.572

Table (2): Sugar content of different honey samples

Sugar content (g/100g) (%)	Darfur	Kordofan	Blue Nile	South Sudan	White honey
Fructose	25.51	25.55	28.24	32.09	32.84
Glucose	1.37	19.82	17.12	24.07	30.60
Sucrose	0.80	1.20	2.85	1.04	1.80
Maltose	-	-	0.91	2.22	-

Table (3): Mineral content analysis of honey samples

mineral content (ppm)	Darfur	Kordofan	Blue Nile	Sudan	White Nile
Na^{+}	13.74	13.25	18.30	6.03	12.00
K^{+}	144.64	58.80	522.22	89.26	17.13
Ca^{+2}	42.02	26.50	38.37	32.17	25.62
Mg^{+2}	18.00	19.74	29.32	31.14	0.62
Fe^{+2}	1.08	0.99	1.81	0.99	3.70
Mn^{+2}	0.48	0.40	0.55	0.44	0.03
Cu^{+2}	0.74	0.08	0.12	0.09	0.01
Co^{+2}	0.000	0.0126	0.0063	0.000	0.000
Zn^{+2}	0.07	0.18	0.25	0.06	0.09

Table (4): Concentration of ascorbic acid

Samples	Concentration (mg/100g)
Darfur	0.9975
Kordofan	0.7895
Blue Nile	0.8925
South Sudan	2.007
White honey	0.7705

One of the fundamental aspects that influence the commercial value of honey is its botanical and geographical declaration of origin.The results obtained for the several physicochemical parameters determined are presented in Tables 1, 2, 3, 4. The pH values of the analyzed honey samples found 4.61 (Darfur), 4.43 (Kurdufan), 4.24 (South sudan),4.76 (Blue Nile amber) and 3.98 (Blue Nile White).

Moisture Percent in he analyzed honey samples found 19.20% (Darfur), 17.20% (Kurdufan), 20.40% (South sudan),19.60% (Blue Nile amber) and 20.00% (Blue Nile White). The water content of honey depends on various factors, like the harvesting season, the degree of maturity reached in the hive and climatic factors. The maximum amount of water contained by honey is regulated for safety against fermentation. The moisture, or conversely the soluble solids in honey, is determined by measuring the refractive index of honey using a refractometer.

The viscosity of honey is affected by moisture content and floral source. Table (1) shows how the viscosity changes as moisture content and floral source change. The viscosity of honey decreases rapidly as moisture content rises.

Honey acidity is due to the presence of organic acids, mainly gluconic acid, in equilibrium with their corresponding lactones. Free acidity was found below 50 mg/kg , indicating the absence of undesirable fermentation except in white honey it was found 146 mg/kg due to the high content of Glucose. The variation in free acidity among different honey types may be attributed to variation due to harvest season. Honey consists mostly of the monosaccharides such as glucose and fructose. The actual proportion of fructose to glucose, in any particular honey, depends largely on the source of the nectar.

The mineral content is an important index of possible environmental pollution and a potential indicator of geographical origin of honey. The results of the cationic metals determined in Sudan honey samples are summarized in Table 3. Potassium was quantitatively the most important mineral. Studies from other geographical locations also

revealed potassium to be the most abundant element [18]. Sodium, calcium and iron magnesium were present in moderate amounts in the honey samples. Manganese, cobalt, copper, and zinc were present in small amounts.

The variation of ascorbic acid concentration is affected by moisture content and floral source and its botanical and geographical declaration of origin.

CONCLUSIONS

The analytical result indicated that honey produced in the Sudan come mainly from nectar and presented excellent quality properties.

The free acidity values were mostly low, indicating honey freshness. In addition, the samples contained 20% water, below the maximum amount allowed by international regulations.There is a significant variation between honey samples. The Blue Nile honey sample was observed to contain the highest level of ash, content, potassium, sodium, and calcium.

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