Sudan University of Science and Technology

College of Agricultural Studies

Department of Food Science and Technology

Chemical and Physico-chemical Characteristics of Bees Honey

الخصائص الكيميائية والفيزيوكيميائية لعسل النحل

A Thesis Submitted to Sudan University of Science and Technology in Partial Fulfillment for the Requirements of B.Sc. in Food Science and Technology

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بسم الله الرحمن الرحيم

قال تعالى: (وأوحَى رَبُك إلى النحْل أن أتخذى منَ الجبَال بُيُوتا ومنَ الشَجَرَ ومما يَعرشونَ * ثُم كلي من كُل الثمرات فاسلكى سُبُل ربك ذُلُلاً يخَرج من بُطُونهَا شَرَابٌ مُختَلف أَلوانُهُ فيه شفَاءٌ للنَاس إن فى ذَالكَ لأيَةً لقوم يَتَفَكرُون)

صدق الله العظيم

سُورَة النحْل الآية (68-69)

DEDICATION

To our parents,

Brothers and Sisters,

Teachers and friends…

With respect.

ACKNOWLEDGEMENTS

*First of all, thank almighty **Allah** who provides us with health and ability to fulfill this work. Words cannot express the especial appreciation and the deepest gratitude a feel our supervisor **prof. Hattim Makki Mohamed Makki** for his kind encouragement, close and valuable supervision as well as his precious advices.*

Thanks are also given to our colleagues and last but not least our great gratitude to our family for their help, advice and continuous encouragement.

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ABSTRACT

The main goal of this research was to study the chemical and physic-chemical characteristics of two local Sudanese bees honey obtained from Khartoum and Darfur markets and to compare them with an imported commercial product and standard specification of the Sudanese Standards and Metrology Organization (SSMO).

The results obtained in this study indicated that, the two local Sudanese bees honey had the higher levels of moisture (19.46% and 18.62 %) and the lower levels of total sugars (67.97% and 70.21%) in comparison with those of the imported bees honey product, which were found to be 16.41% and 72.95%, respectively.

On the other hand, the imported bees honey product was found to have the higher level of reducing sugars (68.57%) and the lower levels of ash (0.86%) and 5-hydroxy methyl furfural (53.51 mg / 100 g), when compared with the two local Sudanese bees honey samples, which were found to range between 62.38% - 63.03%, 0.92% - 0.94% and 55.35 mg / 100 g - 60.82 mg / 100 g, respectively. Moreover, significant differences were found in the different bees honey samples in regard to their total soluble solids contents (T.S.S %). The highest value (80.34%) was recorded for the imported sample. Also, the viscosity of the tow Sudanese bees honey was found to range between 237.02 – 298.27 centipoises in comparison with 258.05 cp for the imported product.

However, when comparing the chemical characteristics of the different bees honey samples with those of local Sudanese bee's honey specifications, except for the reducing sugars and water insoluble solids (W.I.S), all the other chemical characteristics were found to be within the specification of the Sudanese Standard and Metrology Organization (SSMO).

ملخص الدراسة

الهدف الرئيسى من هذا البحث دراسة الخصائص الكيميائية والفيزيوكيميائية لعينتين عسل نحل محليتين مأخوذة من أسواق الخرطوم ودارفور و لمقارنتهما مع منتج تجاري مستورد و المواصفة القياسية من الهيئة السودانية للمواصفات والمقاييس.

النتائج المتحصل عليها في هذة الدراسة تدل على أن، عينتين عسل النحل السودانيتين حأزتا على مستويات عالية من الرطوبة (19.46% و18.63%) و مستويات منخفضة من السكريات الكلية (67.97%و 70.21%) ومقارنتهما مع منتج عسل النحل المستورد والتى وجدت فيه 16.41% و 72.95% على التوالي.

من ناحية آخرى، منتج عسل النحل المستورد وجد به مستوي عالي من السكريات المختزلة (68.57%) و مستوي منخفض من الرماد (0.68%) و 5-هيدروكس ميثايل فورفيورال (53.5l ملجم/100جم)، عندما قورنه مع عينتين عسل النحل المحليتين التى وكانت فى مدى بين 63.03% - 62.38% , 0.94 - 0.92% 60.82, – 55.35ملجم/100جم، على التوالي. علاوة على ذلك وجود إختلاف معنوي في العينات المختلفة لعسل النحل فيما يتعلق بالمواد الصلبة الكلية الذائبة (T.S.S%). و أعلى قيمة (80.34%) التى سجلت للعينة المستوردة. أيضاً اللزوجة لعينتين عسل النحل السودانيتين كانت في المدى ما بين 27 .298 – 237.02 cp سنتبويس مقارنة مع 258.05cp في المنتج المستورد.

لكن، عندما قورنة الخصائص الكيميائية لعينات عسل النحل المختلفة مع المواصفة المحلية لعسل النحل، بأستثناء السكريات المختزله والمواد الصلبة الغير ذائبة في الماء ، كل الخصائص الكيميائية الآخري وجدت ضمن المواصفة السودانية القياسية (SSMO).

CHAPTER ONE

1. INTRODUCTION

1.1 Bees honey

Bees honey is a popular product throughout the world and it is naturally made by bees from nectars extracted from the nectarines of flowers. The product varies in taste, aroma and colour according to its source (**National Honey Board, 2002)**. Also, the composition of bees honey varies depending mainly on the source of the nectar from which is originated and to a lesser extend on a certain external factors such as climatic conditions and bee keeping practices.

It is a fact that, honey possesses nutritional health value and healing properties, therefore, it has an important place in the traditional food preparation in many societies. Bees honey is widely used as a medicine and often regarded as a special tonic food during illness. In Sudan, local farmers harvested bees honey from natural forest but today apiculture is a good industry in many parts of the country **(Adesunkami and Oyelami, 1994)**. However, several international organizations have set specific quality standards and enforce legislation and specifications for this commodity in the different countries.

1.2 Aim of the study

The main goal of this investigation is to compare the chemical and physico-chemical properties of two local Sudanese bees' honey products with those of a commercial imported bees honey with reference to the Sudanese Standards (SSMO).

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Bees honey

2.1.1 Definitions

Honey is a natural sweet substance produced by honey bees 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 is collected by bees, transformed by combining with their specific substances, deposit, dehydrated, stored and left in the honey comb to ripen and mature **(Codex, 1994)**.

Also, Honey is defined as a substance made when the nectar (sweet deposits from plants) is gathered, modified and stored in the honeycomb by honey bees and stipulates a pure product that does not allow for the addition of any other substance. This includes (but is not limited to) water or other sweeteners **(National Honey Board, 2003)**.

Ahmed, *et. al*, (2007) defined honey as a natural complex food product produced by bees from nectar of plants and from honeydew. It is a unique sweetening agent that can be used by humans without processing.

2.1.2 Chemical and physical nature

Honey consists essentially of different sugars, predominantly fructose and glucose as well as other substances such as organic acids, enzymes and solid particles derived from honey collection. The colour of honey varies from nearly colourless to dark brown. The consistency can be fluid, viscous or partly to entirely crystallized. The flavour and aroma vary, but are derived from the plant origin **(Codex, 1981)**.

Honey is a thick, translucent, pale yellow or yellowish brown to dark brown liquid with a sweet and characteristic taste which varies with the floral origin or pasturage and environmental conditions. Apiary honey is either light yellow, yellow ,light amber, amber or dark amber in colour with optical density ranging from 0.32 to 1.0; and a low pollen count contrary to squeezed honey which is either light amber or amber in colour whit optical density of 0.05 to 0.28 and a higher pollen count. Due to high content of pollen, wax and other small water –insoluble particles the squeezed honey is unclean and is rapidly crystallized, fermented and turns brown in color.
It contains chiefly dextrose, moisture small amounts of sucrose and mineral constituents **(Mahindru, 2007)**.

2.1.3 Honey types

The type of honey can be divided according to geographical location. Various studies on honey have shown that there are immense antimicrobial differences within different honeys and this is directly related to the different countries or regions from which they are sourced. The geographical location of the floral origin may affect the photochemical composition and physiology of the floral species which essentially affects the honey that has been harvested from this flora. Diversity of the plant species is dependent on environmental factors such as sunlight, moisture and soil composition. It has been postulated that the difference between countries can be attributed to varying compositions of pollen or nectar which has the greatest effect on honey composition **(Henriques, *et. al.*, 2005; Kaskoniene and Venskutonis, 2010)**.

The previous authors demonstrated that, essential oil composition in honey was dependent on geographical location even when compared within same plant species. Thus, the composition can be substantially different even for honeys of the same

floral origin. This fact was further demonstrated by **Irish, *et. al.* (2011)**, who reported a variation in hydrogen peroxide activity within the equivalent floral species confirming that environmental conditions in different regions play a role in the relationship between floral source and non-peroxide activity. Table (1): shows the different types of bees honey and their physic- chemical characteristics as reported by **Satal (2011)**.

2.1.4 Nutritional value

2.1.4.1 Chemical composition

Honey is primarily made of water and carbohydrates it contains chiefly dextrose, small amounts of sucrose and mineral constituents. Purer honey laevorotatory as it contains more of fructose. Obviously, its fructose: glucose ratio should be greater than one. Also it contains enzymes, vitamins, suspended matter, dextrin, maltose, melezitose, pentose's and gum. **(Mahindru, 2007)**.

Bees honey was also reported to contain a blend of flavonoids and phenolic acids which are considered as antioxidant that eliminate potentially destructive free radicals in human body. The darker honeys tend to have slightly higher antioxidant levels than do lighter varies **(Kumar, *et. al*, 2010)**.

2.1.4.2 Minerals and vitamins

Honey is contains trace amounts of several minerals and vitamins. You can find niacin, calcium, copper, riboflavin, iron, magnesium, potassium and zinc in honey **(Kumar, *et. al,* 2010)**.

Table (1): Different types of bees honey and their physicochemical characteristics

Type	Colour	Flavor	Taste	Texture
Mountain honey	Deep brown	Slirong and characterized	Very sweet	Heavy
Alfalfa honey	White	Light	Sheathy and delicious	Soft granules crystallized
Seder honey	Peal brown	Aromatic	Charalirized taste	Heavy texture
Mountain crown honey	Peal white	Silky and fluffy	S0ft	Granulated clear
Orange blossom honey	Light amber	Light	Testable	Sticky heavy
Alkhouzame honey	Light amber	Aromatic and Testable	Testable	Ointment creamy
Eucalyptus honey	Amber	Aromatic	Relatively clear Testable	Sticky soft crystallized
Chestnut honey	Deep brown	Strong and sharp	Bitter taste	Thick and sticky
Gulf honey	Reddish brown	Strong smell	Sour taste	Stiky-semi liquid
Zarorr honey	Light amber	Aromatic	Sweet and Silky	Soft granulated
Acasia honey	Amber	Light	Very sweet	Liquid

Source: Satal (2011).

The composition of honey is mainly sugars and water. With several vitamins and minerals, including B vitamins Thiamine (B1), Riboflavin (B2), Niacin (B3), Pantothenic acid (B5) Pantothenic acid (B5), Pyridoxine (B6), Folic acid (B9), Ascorbic acid (C) and Phyllochinon (K). The other constituents of honey are amino acids, antibiotic-rich inhibine, proteins, phenol antioxidants, and micronutrients. The sugars in honey are sweeter and give more energy than artificial sweeteners and the most abundant sugar in honey is fructose. These substances are of nutritional and health importance. Some of the vitamins found in honey include ascorbic acid, pantothenic acid, niacin and riboflavin; along with minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc. The high nutritional profile of honey with wide range of nutrients (although in minute quantities), encourages its use as food. Due to the low quantities of some of the NH's essential nutrients, it is advisable for adults to take it Natural honey in large quantities (70 – 95 g daily) to get the full desirable nutritional and health benefits. Growth Food is eaten for nourishment, metabolic activities, growth and healthy living. Regular consumption of natural honey gives all these benefits. In fact, honey is a complete meal. It contains major components of a meal, and micronutrients that will enhance the digestion and absorption of these major dietary components, as well as those required for metabolism and body function **(Ajibola, *et. al*, 2012)**.

As reported by **Joseph (2015)**, bees honey contains variety of vitamins and minerals. The type of vitamins and minerals and their quantity depends on the type of flowers used for apiculture. Commonly, honey contains vitamin C, calcium and iron. These vitamins and minerals which in bees honey are completely absent, in regular sugar from any other source.

2.1.5 Utilizations

2.1.5.1 Industrial food utilization

Honey is used in food industry as sweetening agent, in place of cane sugar for sweetening tea, coffee, milk, buttermilk and other beverages. It is extensively used in breads, cakes, biscuits, pastries, candies, chewing gums, toffees, nuggets and as a spread on bread toast. Canning and preservation of fruits and jams with honey have been extensively practiced. Large, quantities of honey have been used in making of alcoholic drinks as it gives a characteristic odour to port wines. Also, it is used in the preparation of sweet wine by mixing with ordinary wine and brandy **(Mahindru, 2007)**.

2.1.5.2 As medicine

As reported by **Molan (2001)**, honey is used for the treatment of eye disorders. In the Rangarya Medical College of India it has been used to treat corneal eye ulcers, treatments of plepharitis (inflammation of the eye-lids) catarrhal conjunctivitis and keratitits. Also in Russia**,** honey has been applied to the eye under the lower eye lid against chemical and thermal burns of the eye, conjunctivitis and infections of the cornea. The healing effect of honey is explained by its anti-inflammatory, antibacterial and antifungal actions of honey.

Honey is considered as well as medicine. It is usually taken with water to act as a stimulant and helps in replenishing lost energy. It is nonirritating**,** and provides maximum energy with minimum shock to the digestive system. Appreciable increase in the content of red blood corpuscle (RBC) and hemoglobin (Hb) is attributed to the presence of iron, manganese, copper and other elements in honey. Honey -butter is a

mixture of honey and butter/ghee is often recommended for children as substitute for cod liver oil. Honey is also serving as important medicine by virtue of its mild laxative, bactericidal, sedative, antiseptic and alkaline characteristics. Moreover it is generally used as a remedy for cold, cough, fever, sore eyes and throat, tongue and duodenal ulcers, liver disorders, constipation, diarrhea, kidney and other urinary disorders, pulmonary tuberculosis, marasmus, rickets scurvy and insomnia. Besides it is used as a remedy on open wounds after surgery. It has been reported to prevent infection and promote healing, as it has many ingredients very similar to antibiotics. It is also useful in healing of carbuncles, chaps, scalds, whitlows and skin inflammations. In tact germs of typhoid, broncho -preumonia and dysentery have been known to be effectively controlled by consuming honey. Honey is traditionally used as a treatment of mouth ulcers and other infections of the oral mucous membrane **(Mahindru, 2007)**.

Honey is mixed with onion juice and used as a good remedy for arteriosclerosis in brain. The coronary dilatory action of honey is due to the presence of acetyl choline. Diet rich in honey is recommended for infants, convalescents, diabetic, patients and individuals **(Mahindru, 2007)**.

In Australia and New Zealand manuka honey is traditionally used for wound healing, Manuka honey was also approved as a wound dressing in the U.S. **(F D A, 2009)**.

In Egypt, honey as oral rehydration solution was found to promote rehydration of the body and sped recovery from vomiting and diarrhea **(Abdulrhman, 2010)**.

William (2013) indicated that**,** bees honey could be considered as anti-allergic, anti-inflammatory, antibacterial, antioxidant, antiviral, arthritis, autoimmune

protection, eye health, prebiotic, and promotes calcium and selenium absorption as well as wounds healing.

According to **Frances, *et. al,* (2015)**, bees honey is used as a remedy for Chronic kidney infections disease (CKD).

Also, **Bogdanov (2016)** mentioned that, bees honey is used against muscle cramps and acute gastroenteritis in children.

2.1.5.3 As cosmetics

Bees honey constitutes an important ingredient of certain lotions, cosmetic, soaps, palms, toilet, waters and inhalation. Besides honey masks and baths, have been proved to be beneficial to the skin. **(Mahindru, 2007)**.

According to the literature, Queen Cleopatra in the ancient time took a bath of honey and milk for her beauty. Today honey is also used in many cosmetic products such as shampoo, Hair palm, Purifying lotion, honey cream, sun cream, hand lotion, lip balm and honey masks. Generally, honey cosmetics are suitable for all skin types. Bees honey is hygroscopic, antibacterial and fungicide and its ingredients nurture the skin. It is mildly acidic and contributes to strengthening the upper protective skin layer (pH of the skin is 5.5). Honey mask is the best form that complies with the consistency of honey. It nourishes the skin and keeps it moisturized. Regular use of them keeps skin juvenile and retards wrinkle formation (**Bogdanov, 2016)**.

2.1.6 Quality

High-quality honey can be distinguished by its fragrance, taste, and consistency. Ripe, freshly collected, high-quality honey at 20 °C (68 °F) should flow from a knife in a straight stream, without breaking into separate drops. After falling

down, the honey should form a bead. The honey when poured should form small temporary layers that disappear fairly quickly, indicating its high viscosity. If not, it indicates excessive water content (over 20%) of the product **(Bogdanov, 2008)**.

Honey with excessive water content is not suitable for long-term preservation. **(Allan, 2001; and Bogdanov, 2008)**.

Fresh honey in jars should appear as a pure, consistent fluid, and should not set in layers. Within a few weeks to a few months of extraction, many varieties of honey crystallize into a cream-colored solid. Some varieties of honey, including tupelo, acacia, and sage, crystallize less regularly. Honey may be heated during bottling at temperatures of 40-49 °C (104-120 °F) to delay or inhibit crystallization. Overheating was mentioned to affect enzyme activity such as diastase activity **(Bradbear, 2009)**.

2.1.6.1 Quality according to the consumer preferences

For the consumer of honey, the important features of honey are its aroma, flavour, colour and consistency, all of which depend upon the species of plants being visited by the bees. For example, bees foraging on sunflower will produce a golden honey that granulates (crystallizes) quite quickly, while bees foraging on avocado produce a dark honey that remains liquid over a long period. The factors of aroma and flavour of honey are subjective, and honey is often judged according to its colour. Usually dark-coloured honeys have a strong flavour while pale honeys have a more delicate flavour. A great number of different substances (alcohols, aldehydes, organic acids, and esters) contribute to the flavor of honey. These are volatile compounds and evaporate easily at temperatures above 35 _C: this is one of the reasons why honey quality is reduced by heat. It is impossible to give a comparable value to the

subjective values of flavour and aroma: the relative popularity of dark and light coloured honey varies from country to country. Colour can sometimes be a useful indicator of quality because honey becomes darker during storage, and heating. However, many perfectly fresh, unheated and uncontaminated honeys can be very dark **(Bradbear, 2009)**.

2.1.6.2 Quality according to trade criteria

Honey is not a simple commodity with a single standard composition. It is a product that is harvested and marketed in nearly every country. Yet there is no single, international standard for honey quality. Any Nations and market regions set their own criteria for honey, defining what honey is, and what its composition should be. Currently a major issue of concern for the world honey market is the contamination of honeys with the residues of medicines used to control bee diseases. Therefore the EU has the most stringent honey quality requirements: honey is not permitted to contain any trace of antibiotic. In the US, some trace levels of antibiotics are permitted **(Bradbear, 2009)**.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Materials

Samples of honey were obtained from Khartoum markets (Jun 2016). The samples were kept in refrigeration until needed for the different investigations.

3.2 Methods

3.2.1 Chemical methods

3.2.1.1 Moisture content

The moisture content of the different samples was determined according to **person (1997)**.

Five (5 gm ±1mg) sample was accurately weighted and mixed with 20 gm pure quartz sand (500 microns) and the mixture was well mixed and transferred to aluminum flat dish and heated on steam bath for partial drying. After that, the sample was dried in a vacuum oven (25 mm Hg) at 70°C until a constant weight was achieved. Then, the moisture as % was determined according to the fallowing equation:

$$\text{Moisture (\%)} = \frac{(Ws - Wd)}{\text{Sample weight (g)}} \times 100\% \qquad \text{[eq.1]}$$

Where:

Ws = weight of sample before drying.

W_d = weight of sample after drying.

3.2.1.2 Total sugar, reducing and non-reducing sugars

The total sugars as well as reducing and non-reducing sugars were determined according to Lane and Eynon titrometric method as described by the Association of Official Analytical Chemists (**AOAC, 1990)**.

(A) Reducing sugars

A sample of 5 gm ± 1mg was weighted and transferred to 250 ml volumetric flask. 100 ml of distilled water was carefully added and then neutralized with 1.0 N NaOH, about 2 ml of standard lead acetate (NO. 23500, BDH, England) was added and the flask was shaked and left to stand for 10 min. After that, 2 ml of sodium oxalate were added to remove the excess amount of lead acetate and the solution was made up to volume (250 ml) with distilled water and filtered.

(B)Total sugars

From the previous clear sample solution, 50 ml was pipetted into a 250 ml conical flask and 5 gm citric acid and 50 ml distilled water were added slowly. Then, the mixture was gently boiled for 10 min to complete the inversion of sucrose and left to cool at room temperature. After that, the solution was transferred to 250 ml volumetric flask, neutralized with 20% NaOH solution in the presence of few drops of phenolphthalein (NO. 6606 J. T Baker, Holland) until the colour of the mixture disappeared and the sample was made up to volume before titration.

Procedure:

A volume of 10 ml from the mixture of Fehling's (A) and (B) solutions was pipetted into 250 ml conical flask. Then, sufficient amount of the clarified sugars

solution was added from burette to reduce Fehling's solution in the conical flask. After that, the solution was boiled until a faint blue colour is obtained. Then, few drops of methylene blue indicator (S-d-FINE-CHEM LIMITED) were added to Fehling's solution and titrated under boiling with sugars solution until brick-red colour of precipitate cuprous oxide was observed. Finally, the titre volume was recorded and the amount of inverted sugars was obtained from Lane and Eynon Table. The total sugars, reducing and non-reducing sugars were calculated by using the following formulas:

Calculation:

$$\text{Total sugars \%} = \frac{\{\text{invert sugar (mg)} \times \text{dilution factor}\} \times 100\ \%}{\text{Titre volume (ml)} \times \text{sample weight (g)} \times 1000}$$

[eq.2]

$$\text{Reducing sugars \%} = \frac{\{\text{invert sugar (mg)} \times \text{dilution factor}\} \times 100\%}{\text{Titre volume (ml)} \times \text{sample weight (g)} \times 1000}$$

[eq.3]

$$\text{Non-reducing sugars \%} = \{\text{Total sugars (\%)} - \text{reducing sugars (\%)}\}$$

Where: Titre volume = (Sample – blank)

[eq.4]

3.2.1.3 Determination of hydroxy methyl furfural

The hydroxy methyl furfural content was determined in each sample as mentioned by **person (1997)**.

Principle:

A colour is developed when hydroxy methyl furfural (HMF) is reacting with p-toluidine and barbituric acid during heating, so the sample must not be subjected to heat.

Procedure:

A sample 10 gm of honey was dissolved without heating in 20 ml oxygen – free distilled water. The sample was transferred to a 50 ml graduated flask and make up to volume (honey solution). Then 2.0 ml of honey solution was pipette into two (2) test tubes (25 mm diameter) and 5.0 ml p-toluidine solution was added to each tube. After that,1 ml water was added into one test tube and into the other 1ml barbituric acid solution was added and both of them were well shacked. The tube with added water was served as a bank. Then, the absorbance of the sample against the blank was immediately read at 550 nm using a 1cm cell.

The amount of HMF in micrograms in the sample was determined from a standard curve prepared under the same conditions.

3.2.1.4 Determination of Ash

The ash content was determined according to the method described by the **(AOAC, 2003)**.

Principle:

The inorganic materials which are varying in concentration and composition are customary determined as a residue after being ignited at a specified heat degree.

Procedure: A sample of 5g ± 1mg was weighed into a pre-heated, cooled, weighed and tarred porcelain crucible and placed into a Muffle furnace (No.20. 301870, Carbolite, England) at 600 °C until a white gray ash was obtained. The crucible was transferred to desiccators, allowed to cool to room temperature and weighed. After that, the ash content was calculated as a percentage based on the initial weight of the sample.

Calculation:

$$\text{Ash (\%)} = \frac{[(\text{Wt of crucible} + \text{Ash}) - (\text{Wt of empty crucible})]}{\text{Initial weight of sample (g)}} \times 100\ \%$$

[eq.5]

3.2.2 Physicochemical characteristic

3.2.2.1 Determination of hydrogen ions concentration

The hydrogen ions concentration (pH) in each sample was determined as mentioned by **Agbagwa*, et al* (2011)**.

Procedure:

After standardization of the pH -meter with two buffer solutions (pH of 4.0 and 7.0), the electrode of the pH –meter was rinsed with distilled water, immersed in the sample solution (20 °C) and left to stand until a staple reading was achieved. All the readings were expressed as pH to the nearest 0.01 pH units.

3.2.2.2 Determination of total soluble solid

The total soluble solids as percent (T.S.S %) in the different samples were measured as described by **Ranganna (2001)**.

Principle:

The index of refraction of a substance is a ratio of light velocity under vacuum to its velocity in the substance which is largely dependent on the composition, concentration and temperature of the sample solution.

Procedure:

After the adjustment of the Hand-Refractometer (No.002603, BS-eclipsprism, the prism was closed and the reading was recorded to the nearest 0.01 as T.S.S %.

3.2.2.3 Determination of viscosity

The viscosity was measured in the different honey samples by using a vescoanlizer as declared by **person (1997)**.

3.2.3 Statistical analysis method

The data obtained in this study were subjected to Statistical Analysis by using the Statistical Package for Social Science (SPSS). The mean values were obtained by the Analysis of Variation (ANOVA). Probability of 5% was used to indicate the significances according to Duncan's Multiple Range Test (DMRT) as described by **(Mead and Gurow, 1983)**.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Chemical characteristics of Sudanese bees honey in comparison with imported product

Table (2) shows the Chemical characteristics of two local Sudanese bees honey in comparison with imported commercial bees honey product the results obtained in this study indicated that the two local Sudanese bees honey that purchased from Khartoum and Darfur markets had the higher moisture levels (19.46% and 18.63%) and total the lower total sugars content (67.97% and 70.21%) in comparison with those of imported bees honey product (C), which were found to be 16.41% and 72.95%, respectively.

In contrast, the imported bees honey product (C) had the higher levels of reducing sugars (68.57%) and the lower levels of ash (0.86%) and 5-hydroxy methyl furfural (53.51 mg /100g) when compared with the two found to range between 62.38% - 63.03%, 0.92% - 0.94% and 55.35 – 60.82 mg / 100g, respectively.

The results obtained in this study for the two Sudanese honey samples are wee agree with those reported **Muas (2013)**. However, **Mahindrn (2007)** and **Ajibola, *et. al*. (2012)** mentioned that, bees honey is primarily mad of water and carbohydrates. It contains chiefly dextrose, small amounts of sucrose and its fructose: glucose ratio is greater than one.

Table (2): Chemical characteristics of two Sudanese Bees honey in comparison with imported commercial product.

Chemical characteristics	**Sudanese bees honey**		**Imported bees honey**
	(A)	(B)	(C)
	[n = 3 ± S D]		
Moisture (%)	$19.46^{a} \pm 0.13$	$18.63^{b} \pm 0.17$	$16.41^{c} \pm 0.15$
Total sugars (%)	$67.97^{c} \pm 0.32$	$70.21^{b} \pm 0.09$	$72.95^{a} \pm 0.27$
Reducing sugar (%)	$62.99^{b} \pm 0.11$	$63.03^{b} \pm 0.25$	$68.57^{a} \pm 0.98$
Sucrose (%)	$4.98^{b} \pm 0.42$	$7.18^{a} \pm 0.17$	$4.38^{b} \pm 0.86$
5. HMF (mg/100g)	$60.82^{a} \pm 0.42$	$55.35^{b} \pm 0.06$	$53.51^{c} \pm 0.57$
Ash (%)	$0.94^{a} \pm 0.03$	$0.92^{a} \pm 0.01$	$0.86^{b} \pm 0.01$

A ≡ Bee honey sample from Khartoum.

B ≡ Bee honey sample from Darfur.

C ≡ Bee honey sample from Ethiopia.

S.D ≡ Standard deviations.

n ≡ Number of independents determinations.

Mean ± S.D value(s) bearing different superscript letter (s) in each row are significantly different ($P \leq 0.05$).

5. HMF ≡ 5.Hydroxy methyl furfural.

4.2 Physico-chemical properties of Sudanese bees honey in comparison with imported product

Table (3) presents the physico- chemical characteristics of two Sudanese bees honey in comparison with imported bees honey product. From the results obtained in this study, no marked differences were found between the different samples with respect to their hydrogen ions concentration (pH), which was found to range between 4.02 and 4.64. The highest pH value was recorded for the local Sudanese honey sample that obtained from Darfur state. While, the lowest pH value was found in the imported sample.

In contrast, significant differences ($P \leq 0.05$) were found between the different samples in regard to their total soluble solid contents (T.S.S %). The highest value (80.34%) was recorded for the imported honey sample, while the lower value (70.20%) was found in bees honey sample that obtained from Khartoum state.

In addition to that, the viscosity of the two Sudanese bees honey samples was found to range between 237.02 – 298.27 centipoises, compared to 258.05 cp recorded for the imported product. **Bradbear (2009)** declare that, the consistency and other imported features of bees honey such as colour, odor and flavour are mainly depend upon the plant species that visted by the bees.

4.3 Chemical characteristics of two Sudanese bees honey and imported commercial product in comparison with local Sudanese specifications

Table (4) compares the chemical properties of two local Sudanese honey samples and imported sample from Ethiopia country, with those of local Sudanese specifications (SSMO). The results revealed that, except for the reducing sugars and water insoluble solid (W.I.S) contents in local and imported bees honey samples,

Table (3): Physico-chemical characteristics of two Sudanese bees honey in comparison compared with imported bees honey product.

Physicochemical characteristics	**Sudanese bees honey**		**Imported bees honey**
	(A)	(B)	(C)
	[n = 3 ± S D]		
Hydrogen ions concentration (pH)	$4.04^{a} \pm 0.15$	$4.64^{a} \pm 0.30$	$4.02^{a} \pm 0.31$
Total soluble solid (T.S.S %)	$70.20^{c} \pm 0.86$	$75.19^{b} \pm 0.41$	$80.34^{a} \pm 0.24$
Viscosity(cp)	$298.27^{a} \pm 0.95$	$237.02^{c} \pm 6.48$	$258.05^{b} \pm 1.25$

A ≡ Bee honey sample from Khartoum.

B ≡ Bee honey sample from Darfur.

C ≡ Bee honey sample from Ethiopia.

S.D ≡ standard deviations.

n ≡ Number of independents determinations.

Mean ± S.D value(s) bearing different superscript letter(s) in each raw are significantly different ($P \leq 0.05$).

Cp ≡ centipoises.

all the other chemical characteristics of the Sudanese Standard and Metrology Organization (SSMO).

Both the reducing sugars and water insoluble solids contents in the different bees honey samples are not in accordance with the local Sudanese specification as shown in table (4).

Table (4): Chemical characteristics of two Sudanese Bees honey and imported commercial product in comparison with local Sudanese specification (SSMO).

Chemical characteristics (%)	**Sudanese bees honey**		**Imported bees honey**	**SSMO specifications**
	(A)	(B)	(C)	
	[n = 3 ± S D]			
Moisture	$19.46^{a} \pm 0.13$	$18.63^{b} \pm 0.17$	$16.41^{c} \pm 0.15$	≥ 20
Reducing sugars	$62.99^{b} \pm 0.11$	$63.03^{b} \pm 0.25$	$68.57^{a} \pm 0.98$	≥ 60
Sucrose	$4.98^{b} \pm 0.42$	$7.18^{a} \pm 0.17$	$4.38^{b} \pm 0.86$	≥ 10
5.HMF(mg/100g)	$60.82^{a} \pm 0.42$	$55.35^{b} \pm 0.06$	$53.51^{c} \pm 0.57$	≥ 80
Ash	$0.94^{a} \pm 0.03$	$0.92^{a} \pm 0.01$	$0.86^{b} \pm 0.01$	≥ 1
W.I.S (wax)	$1.24^{c} \pm 0.04$	$5.47^{b} \pm 0.12$	$7.83^{a} \pm 0.11$	≥ 0.1

A ≡ Bee honey sample from Khartoum.

B ≡ Bee honey sample from Darfur.

C ≡ Bee honey sample from Ethiopia.

S.D ≡ Standard deviations.

n ≡ Number of independents determinations.

Mean ± S.D value(s) bearing different superscript letter(s) in each samples bees honey are significantly different ($P \leq 0.05$).

5. HMF ≡ 5.Hydroxy methyl furfural.

W.I.S ≡ water insoluble solids.

SSMO ≡ Sudanese Standards and Metrology Organization.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From the results obtained in this study it can be concluded that, both of the two local Sudanese bees honey and the imported commercial bees honey product are found with high quality especially for their moisture, 5-hydroxy methyl furfural, ash and hydrogen ions concentration (pH). The reducing sugars % and water insoluble solids (W.I.S) in the different bees honey samples are not in accordance with the Sudanese specifications of the (SSMO).

5.2 Recommendations

1- More attention should be taken during collection, storage, selling of bees honey to avoid chemical and microbial contaminations.

2- Bees honey should be stored in the fridge or in a cold place to prevent the chemical and enzymatic reactions.

3- Any other investigations in the field of bees honey production will be of great values for the Sudanese honey producers, traders and consumers should be carried.

4- A national survey should be conducted to determine the different types and quality of bees honey.

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