

Physicochemical Properties of Honey From Different Floral Sources

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DEDICATION

This humble effort is dedicated with all love and gratitude to our prophet Mohamed (Peace and Blessing be upon him). Through whom Allah the Almighty says in Glorios Quran.

And to:

My Father

My Mother.....

My Brother and Sisters.

With love
With love

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ABSTRACT

This study was done to investigate the nutritive value of Sudanese bee honey collected from different floral sources (sunut, sidir, talih and sunflower). Physical and chemical analysis were conducted. The results showed significant variation between the different honey types according to the floral origin, which indicate that the level of refractive index ranged from 1.4923 to 1.5038, pH value (4.46 – 5.00), moisture (13.2 – 17.6 %), Ash (0.35 – 0.78 %), protein (0.74 – 1.25 %), ascorbic acid (7.8 – 14.9 mg / 100g), total polyphenols (83.7 – 121.4 mg / 100g), total sugars (76.1 – 81.5 %), reducing sugars (72.6 – 79.3 %), fructose (37.2 – 47.9 %), glucose (25.9 – 40.5 %), sucrose (2.1 – 3.6 %) and free acidity (37 – 63.7 meq / kg).

The concentration of minerals in (ppm) such as potassium were ranged from 1774.5 to 3318.25, sodium (438.90 – 858.70), phosphorus (70.80 – 102.70), iron (39.80 – 50.20), magnesium (17.50 – 37.70), calcium (9.60 – 24.50), manganese (1.95 – 11.70), copper (3.80 – 5.11) and zinc (2.70 – 4.01).

الخواص الكيميائية والفيزيائية للعسل من مصادر مختلفة

هناء يوسف عبدالعزيز حسين

المستخلص

فى هذه الدراسة تم تقدير القيمة الغذائية لأربعة أصناف من عسل نحل سوداني من مصادر نباتية مختلفة شملت (سنط - سدر - طلح و زهرة الشمس) . أجريت عليها التحاليل الفيزيائية و الكيميائية . النتائج المتحصل عليها أظهرت اختلافا معنويا فى التركيب الكيميائي ومن ثم فى القيمة الغذائية لأصناف العسل المختلفة . حيث ان محتوى العسل من العناصر الغذائية يقع فى المدى : -

معامل الانكسار (1، 4923 - 1، 5038) ، الأس الهيدروجيني (4، 46 - 00) ، الرطوبة (13، 2 - 17، 6 %) ، الرماد (0، 35 - 0، 78 %) ، البروتين (0، 74 - 1، 25 %) ، فيتامين "ج" (7، 8 - 14، 9 ملجرام / 100 جرام) ، المركبات الفينولية (83، 7 - 121، 4 ملجرام / 100 جرام) ، السكريات الكلية (76، 1 - 81، 5 %) ، السكريات المختزلة (72، 6 - 79، 3 %) ، الفركتوز (37، 2 - 47، 9 %) ، الجلوكوز (25، 9 - 40، 5 %) ، السكروز (2، 1 - 3، 8 %) و الحموضة الحرة (37، 0 - 63، 7 ملمكافئ /كجم) .

تركيز العناصر المعدنية فى عينات العسل (جزء من المليون) :

البوتاسيوم (1774، 50 - 3318، 25) ، الصوديوم (438، 90 - 858، 70) ، الفسفور (70، 80 - 102، 70) ، الحديد (39، 80 - 50، 20) ، الماغنيزيوم (17، 5 - 37، 70) ، الكالسيوم (9، 60 - 24، 50) ، المنجنيز (1، 95 - 11، 70) ، النحاس (3، 80 - 11، 50) و الخارصين (2، 70 - 4، 01) .

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CHAPTER ONE

INTRODUCTION

Honey is a remarkably complex natural liquid which reported to contain at least 181 substances. The composition of honey is rather variable and primarily depends on the floral source, however, certain external factors also play a role in honey composition, such as seasonal and environmental factors and processing.

Honey is a supersaturated mixture, contains a wide range of minor constituents such as organic acids, amino acids, proteins, minerals, vitamins, enzymes and volatile substances which responsible for the characteristic flavour.

Honey has been used for a long time in a tradition way, it is well known for its beneficial actions such as :

- 1- Antioxidant activity, due to the presence of phenolic compounds, which can inhibit or delay the oxidation and prevention of many diseases.
- 2 - Antimicrobial activity which has been extensively reviewed up to date. The inhibitory activity has been attributed to several key properties of honey including osmotic effect, naturally low pH and production of hydrogen peroxide.
- 3 – Honey has been a valuable survival food for people. It gives remarkable calories for the body and can be easily digested and more palatable.

Due to increasing in international interesting honey characterization, many studies have been carried out in relation to physicochemical parameters. Currently characterization of honey by means of chemical

and sensory characteristics has received several attentions. Vast countries produce honey from various floral sources with wide variations in physicochemical and functional characteristics.

In Sudan very few investigations have been done in Sudanese bee honey, and honey has been investigated as a therapeutic agent by several researchers, but nutritive value of honey is not studied thoroughly.

There is a wide diversity of ecological and geographical floral source, and bee honey is known to be influenced in quality by the type of flowers among other factors.

The present research was directed to investigate the nutritional value of four types of Sudanese bee honey.

The objective of this study was therefore to evaluate the nutritive value of honey collected from different floral sources, to correlate between nectar and pollen sources and the quality and nutritive value of the honey obtained.

CHAPTER TWO

LITERATURE REVIEW

2.1 Honey bee

Honey bee now live in all part of the world except the extreme polar regions. Until the 16th century they were confined to the old world, where they had evolved and were widely distributed long before man appeared on the earth. In the honey bee as in other social animals the colony rather than the individuals of which it is composed in the unit.

2.2 Classification of honey bee

Honey bee is classified as follows :

kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Hymenoptera

Family: Apidae

Scientific name: *Apis mellifera*

2.3 Definition of honey

Honey is the natural sweet substance produced by the worker of honey bee (*Apis mellifera*) from the nectar of plants or from secretions of living parts of plants, which the bee collect, transform and combine with the specific substances and store in the honey comb to ripen and mature (Codex, 1969).

2.4 Floral source

When a bee visit a flower, it comes in to more or less close contact with another according to the structure of the flower. Honey is made

by bees and their raw material for nearly all the worlds supply of honey is nectar produced in the nectaries of flowers.

Nectar is an aqueous sugar containing secretion of plant gland called nectaries which can be found in any part of the plant. Nectar consists in the main of an aqueous solution of various sugars. Other substances such as nitrogen compounds, minerals, organic acids, vitamins, pigments and aromatic substances. The ash content is 0.023-0.45 %, nectar shows an acid reaction (pH:2.7- 6.4). Apart from ascorbic acid (vitamin C) which occurs in appreciable quantities in the nectar of certain plants, there are difference in quality, too, and most of the variations between honeys originate in differences between the nectars from which the honeys are derived.

2.5 Pollen grains

Pollen grains, the male reproductive cells of higher plants, as a rule appear as single cells but they are often found in groups. The identification of the pollen grains is based on their size and shape. Pollen has an exceptionally high vitamin content specially the water soluble vitamins. The B-complex vitamins plus inositol and ascorbic acid are present in pollen (Haydak *et al.*, 1944). The pollen analysis is used for identifying honey sources. Bees are by far the most important pollination insects (Crane, 1972).

2.6 Physical properties

2.6.1 Refractive index

The primary interest in this property of honey is to provide a rapid, accurate and simple measure of moisture content. Early workers, (Bryan, 1908) noted that the moisture values obtained when

converting refractometric reading by mean of tables were higher by 1-2 % moisture content than those from vacuum drying.

Pearce and Jegard (1949) have calibrated such a refractometer against A.O.A.C. drying and reported that the refractometer is much more convenient than the A.O.A.C. vacuum oven method, but not appreciably more accurate. According to Lazaridou *et al.* (2004) the refractive index was ranged between 1.4892 – 1.5043. While, a study carried by Ouchemoukh *et al.* (2007) showed that the refractive index of honey ranged between 1.4889 – 1.4999.

2.6.2 pH value

All of acids have in common the dissociation in aqueous solution to release protons or hydrogen ions. This gives the sourness and other characteristics of acids. A measure of the total concentration of hydrogen ions provides information on the strength of acidity. The pH of a honey sample is not directly related to the free acidity because of the buffering action of various acids and minerals present. White *et al.* (1962) found that honey pH ranged between 3.42 and 6.10 with an average value of 3.91. While Terrab *et al.* (2004) found a range of 3.56 – 4.79. Serrano *et al.* (2004) found that honey pH ranged between (3.72 and 4.64) with a mean (4.07). Moreover Downey *et al.* (2005) reported that honey pH value ranged between 3.75 and 4.61 with a mean of (4.1). Ouchemoukh *et al.* (2007) found that honey pH ranged between 3.49 and 4.43.

2.7 Chemical composition

2.7.1 Moisture content

Honey moisture is the quality criterion that determines the capability of honey to remain stable and to resist spoilage by yeast fermentation. The moisture content of honey may change after removal from the hive as a result of storage conditions after extraction (Bauer, 1960). The control of the water content is an important requirement of the proposed Codex Alimentarius Standard for Honey (1969), which sets an upper limit for moisture of 21% for honey in general.

White *et al.* (1962) Obtained in the analysis of 490 samples of U. S. honey representing single floral types of unknown composition an average of 17.2 % and a range of 13.4 to 22.9 %. Moisture content of 45 samples of Sudanese honey was studied by Ibrahim (1985) who found a range from 13.1 % to 26.8 % with an average of 19.9 %. The moisture content of 25 honey samples from Spain evaluated by (Terrab *et al.*, 2004), and were ranged between 14.2 and 19. The moisture content of 33 Greek honeys from different botanical and geographical origin reported by Lazaridou *et al.* (2004) were ranged between 13.0 and 18.9 %.

The characterization of two types of honey was studied on the basis of their physico-chemical properties, by Serrano *et al.* (2004) who found the range of water content between 14.9 and 18.6 % and a mean of 16.6 %. Downey *et al.* (2005) reported that the moisture content of fifty honey samples from the Island of Ireland, collected over two consecutive harvest seasons, ranged from 15.6 to 20.6 % with an average of 17.6 %. Recently Özcan *et al.* (2006) reported that the moisture content of natural honey was 15.36 %. While Finola *et*

al. (2007) reported that the moisture content of many Argentina honey samples ranged between 16.0 – 23.4 % and the average was 18.4 %. And Ouchemoukh *et al.* (2007) found the moisture content of some Algerian honeys to range between 14.64 and 19.04 %.

2.7.2 Free Acidity

The acids present in honey contribute significantly to its characteristic flavour and its stability towards micro-organisms (White, 1975). Nelson and Mottern (1931) isolated and positively identified acetic, butyric, citric, malic, succinic and formic acids in honey. Stinson *et al.* (1960) reported gluconic acid as the most important honey acid, probably arising from the oxidation of glucose by the naturally occurring enzyme glucose oxidase. 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 (Codex, 1969). Ibrahim (1985) noted great variations in the acidity in the wide collected honey samples from Sudan. The acidity was varied from 6 – 171 meq /kg. Cossta *et al.* (1999) found that the mean value for acidity in different Zulian regions lay in the range of 24.4 – 53.3 meq /Kg. Terrab *et al.* (2004) found that the free acidity ranged between 17.59 – 39.81 meq /Kg with an average of 27.2 meq /Kg. Moreover free acidity ranged between 9.2 – 41.51 meq /Kg and the mean 22.49 meq /Kg was reported by Serrano *et al.* (2004) and ranged from 17.1 – 50.9 meq / Kg with a mean of 32.7 meq / Kg as reported by Downey *et al.* (2005). Also it ranged from 11.9 to 29.4 meq / Kg with an average 20.6 meq / Kg as stated by Finola *et al.* (2007).

2.7.3 Ash content

The ash content is a quality criterion for honey origin. The ash content of honey depends on the material gathered by the bee during foraging, nectar normally has a low ash content. White *et al.* (1962) reported the range of 0.02 to 1.03 % and an average of 0.169% ash. Terrab *et al.* (2004) found that the ash content ranged from 0.16 to 0.60 % with an average of 0.32 %. However, Shin and Ustunol (2005) determined the ash content of three samples of honey from different floral sources and found that the average ash content was 0.3 %. Özcan *et al.* (2006) found that the ash content was 0.177 %, and later study by Ouchemoukh *et al.* (2007) showed a range between 0.06 – 0.54 %.

2.7.4 Protein content

It has been known for many years that honey contains protein materials. Interest in protein content was used to distinguish natural honey from artificial mixture and blend. Schuette and Templin (1930) Studied the United States honey and found that the protein content ranged between 0.25 and 0.76 %. According to Ibrahim (1985) the protein content was 0.532 %. Moreover, Ischayek and Kern (2006) on a study on four types of honey produced in the United State, reported an average of 0.46 % protein content.

2.7.5 Carbohydrate content

Carbohydrates represent the largest portion in honey. The sugars are responsible for much of the physical nature of honey such as viscosity, hygroscopicity, granulation properties and energy values.

Fructose and glucose together account for 85 – 95% of honey carbohydrates, the proposed Codex (1969) standard for honey requires a minimum reducing-sugar content of 65% for flower honeys, both fructose and glucose are defined as reducing sugars. Other sugars present in significant amounts are the disaccharide sucrose (cane sugar), maximum sucrose content of 5% is required by proposed Codex (1969). Sucrose is defined as non-reducing, which is hydrolysed either by mineral acids or by enzyme invertase, each molecule combines with a molecule of water, and fructose and glucose are formed in equal quantities (Walker, 1917). According to White *et al.* (1962) fructose, glucose and sucrose were ranged between 27.2 and 44.3 %, 22.0 and 40.7% then 0.2 and 7.6% respectively. Ibrahim (1985) studied the total sugars, reducing sugar and sucrose and reported values of 60.6 – 79.4 %, 57.8 – 75.7 % and 0.6 – 10.5% respectively. Lazaridou *et al.* (2004) found that the range of fructose and glucose were 22.1 – 41.3 % and 13.5 – 36.3% respectively. Moreover, Serrano *et al.* (2004) noted the range of 10.69 – 45.25 % glucose, 13.55 – 45.0% fructose, 0.14 – 11.49% sucrose and 80.0 – 83.5% total sugars. While Shin and Ustunol (2005) reported values of 37.7% fructose, 35.3% glucose and 2.5% sucrose. Recently, Ischayek and Kern (2006) working on four varieties of honey in the United States, found that the averages of total sugars, glucose, sucrose, fructose content were 85.9 %, 31.3 %, 2.7 % and 36.7% respectively. Also Ouchemoukh *et al.* (2007) reported ranges of total sugars (71.25 – 84.25%), reducing sugars (67.83 – 80.25%), sucrose (0.08 – 5.31%) and glucose (29.4 – 42.0%). While Finola *et al.* (2007) reported (24 – 39.7%) glucose and (33 – 48.4%) fructose.

2.7.6 Ascorbic acid content (Vitamin C)

Pollen grains and bee bread contain small amounts of the vitamins A, K and E (Dadd, 1973). Kitzes *et al.* (1943) assayed considerable numbers of honeys for thiamin (B1), riboflavin (B2), ascorbic acid (C), pyrodoxine (B6), niacin and pantothenic acid, and few for biotin and folic acid.

Griebel (1938) reported 160 – 280 mg /100g of ascorbic acid for mint honey and 7 – 22mg /100g for others. Rosenberg (1942) found an average of 4.9 mg /100g for honey, noting that the vitamin was relatively stable in honey. Werder and Antener (1938) found an average of 1.1 – 14.6 mg /100g for 19 honey samples. Haydak *et al.* (1942) reported an average value for 67 honeys of 3.2 mg /100g honey.

Rahmanian *et al.* (1970) have found high ascorbic acid values (118 -240 mg /100g) for three samples of honey of unknown source from the mountainous Damavand area in Iran. Also they reported an ascorbic acid requirement of 3 – 6 mg / day for the animals they used, and concluded that the honey actually contained 75 - 150 mg /100g of vitamin (C). They suggested the possibility of encouraging the use of honey from this region as a means of helping to relieve the marginal vitamin (C) deficiency.

2.7.7 Minerals content

In addition to the macronutrients and vitamins the living organisms also require mineral elements such as sodium, potassium, calcium and iron in variable amounts for normal body functions and normal growth. Some elements are needed in relatively large amounts and these are sodium, potassium, calcium, phosphorus, magnesium and

sulfur. Others such as iron, copper, manganese, zinc and iodine are needed in only very small amounts and therefore they are termed trace elements. Minerals are present in food at low but variable concentrations and in multiple chemical forms, and they have important biochemical and nutritional functions (Miller and Whistler, 1996). House (1961) pointed out that bees undoubtedly do not collect minerals separately but collect them indirectly along with pollen, nectar and water. Pollen is a rich source of minerals ranging from 2.9 – 8.3 % (Haydak *et al.*, 1942).

Grigoryan *et al.* (1971) reported the presence of 27 trace elements in pollen and honey bee. The predominating mineral element in honey is potassium. Other principal elements found in honey are sodium, calcium, magnesium, iron, copper, manganese, chlorine, phosphorus, sulfur and silica (White and Rudyj, 1978). Ibrahim (1985) in review of Sudanese honeys reported that dark honey being more beneficial than light honey probably due to its minerals content; iron was thought to be the responsible factor. However the effect was also thought to be due to manganese, copper or phosphorus. Ankalm (1998) concludes that dark honeys have a higher mineral content than pale ones, in general K, Ca and P show highest levels with average concentration ranged between 639 – 1845, 111 – 257 and 63.8 – 143 ppm respectively. The qualitative of 25 honey samples from Spain were analyzed by Terrab *et al.* (2004) and reported the range in (ppm): Ca (110 – 248), K (261 – 1380), Mg (37 – 139), Na (256 – 501), P (26 – 96) and S (19 – 43).

Different types of honey studied by Hernandez *et al.* (2005) produced in the Canary Islands were characterized on the basis of their mineral contents. Overall 7 minerals were determined (Fe, Cu, Zn, K, Na, Mg,

and Ca) and the range of these minerals in mg/kg was (3.78 – 7.64), (0.20- 0.44), (1.18 – 2.43), (122 – 1353), (32.7 – 89.6), (28.4 – 49.6), and (57.3 – 82.0) respectively. Özcan *et al.* (2006) reported that the average of Ca, Fe, Mg, Mn, Na, P and Zn content in µg/g was 532, 80.3, 212, 0.848, 354, 436 and 2.94 respectively.

2.7.8 Polyphenols content

Polyphenols constitute one of the most numerous and ubiquitous groups of plant metabolites and are integral part of both human and animal diet ranging from simple phenolic molecules to highly polymerized compounds. Polyphenols traditionally have been considered anti – nutrient by nutritionists because of the adverse effect of tannins (one type of polyphenols), on protein digestibility (Bravo, 1998). Polyphenols can form complexes with metal cations through their carboxylic and hydroxylic groups, and thus interfere with intestinal absorption of minerals (Brune *et al.*, 1989). Iron may not be bioavailable because of the negative influence of both phytate and tannin on iron absorption and nutrient digestibility. Considerable research has confirmed that iron deficiency within population groups is not only caused by the inadequate iron intake but also by interactive factors adversely affecting dietary iron bioavailability (Patwardhan, 1961). Tannins decrease protein digestibility, either by inactivating digestive enzymes or by reducing the susceptibility of the substrate proteins after forming complexes with tannins and absorbed ionizable iron (Reedy *et al.*, 1985). Browne (1908) noted that of the 92 honeys he analyzed, 25 gave a positive test for phenolic compounds with ferric chloride, the 5 most intense reactions being from very dark honeys. Oxidation of these compounds can lead to coloured material,

the development of colour in honey in storage has been ascribed by Milum (1939) to several factors : combination of tannins and other polyphenols with iron from containers and processing equipments, reaction of reducing sugar with substances containing amino acids, and instability of fructose in acid solution (caramelization).The antioxidant activity of honey, however, varies greatly depending on the honey floral source (Frankel *et al.*, 1998). The antioxidant activity of phenolic compounds might significantly contribute to the human health benefits of plant food, which are generally a known legal to be of considerable importance because of it chemoprotective effecting human beings (Bravo, 1998). In the past several years there has been increasing evidence of the antioxidant capacity of honey. Honey has therefore great potential to serve as natural food antioxidant. Yaoa *et al.* (2003) analyzed phenolic acids of Australian and Newzealand honeys by HPLC and found that the mean content of the total phenolic acids in honey was 5.14 mg/100g. These results showed that phenolic acids could be used for honey floral origins. Similarly Yaoa *et al.* (2004) studied seven phenolic acids related to the origins of nine monofloral "Eucalyptus" honeys from Austaralia. They showed that total phenolic acids ranges from 2.14 – 10.3 mg/100g, this result indicates that the species – specific difference can also be found in the honey profiles of phenolic acids. Also Yaoa *et al.* (2005) studied Australian honeys from 5 botanical origins. The phenolic compounds present in these honeys ranged from 2.13 – 12.11 mg/100g. While Meda *et al.* (2005) reported that several honey samples (27) from Burkina faso were analyzed to determine that their total phenolic contents varied considerably with the highest values obtained for honey. Total phenolic content mg/00g varied from 32.59 to 114.75 mg

/ 100g with mean of 74.38 mg /100g. Recently Ouchemoukh *et al.* (2007) found the range of polyphenolic compounds in some Algerian honeys to be between 64 to 130.4 mg/100g.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

The experimental material in the present study consisted of four samples (from H₁ to H₄) of crude unprocessed Sudanese bee honeys, collected from different floral sources. All samples were collected in August 2006 from bee keepers and stored in a refrigerator (4°C ± 2°C) in an air tight plastic containers during analysis, the tests were performed within 5 months following collection. The code of the type of honey samples, as well as the family, the local and scientific name of the plants that form the basic flora of the honey samples are shown in Table (3.1).

Table 3.1 Floral sources of honey samples studied:

Code	Local name	Family	Scientific name
H ₁	Sunut	Mimosaceae	<i>Acacia nilotica</i>
H ₂	Sidir	Rhamnaceae	<i>Zizyphus spina –christi</i>
H ₃	Talih	Mimosaceae	<i>Acacia seyal</i>
H ₄	Sunflower	Compositae	<i>Helianthu annuus</i>

3.1.1 Sampling

Sample free from granulation was mixed thoroughly by stirring or shaking, the granulated sample was placed in close container in water – bath and was heated 30 min at 60°C – 65°C until liquefied. The sample was shaken occasionally and mixed thoroughly and cooled rapidly as soon as sample liquefies. The foreign matter such as wax, bees, particles of comb, etc, which was present the sample was

heated to 40° C in water – bath and was strained out through cheese–cloth in hot–water funnel before sampling (Codex, 1969).

3.2 Methods

3.2.1 Pollen grain analysis

Pollen grains analysis was carried out using the method established by the International Commission of Bee Botany described by Louveaux *et al.* (1978). Ten grams of honey were weighed and dissolved in 20 ml of hot water (not more than 40°C). The solution was centrifuged for 10 minutes at 5000 rpm and the supernatant liquid was decanted and the sediment was colored by basic fuchsin dye and put on a slide. After drying the sediment was mounted with glycerin, covered with a cover slip and examined under a light microscope.

3.2.2 Physical Analysis

3.2.2.1 Refractive index

Refractive index of the test sample was determined by Abbe-refractometer (Hilger, 27137) at a constant temperature (20° C). The determination was carried out at (30°C), so that the reading was converted to standard temperature of (20° C) according to the temperature corrections.

Temperature corrections " Refractive index "

- Temperature above 20°C → 0.00023 per ° C was added.
- Temperature below 20° C→ 0.00023 per ° C was subtracted.

3.2.2.2 pH value

pH value was determined according to A.O.A.C (1984), using pH meter. Ten grams of honey weighed accurately and diluted in 75 ml distilled water. The pH electrode was immersed in the honey solution and the pH value was recorded.

3.2.3 Chemical Analysis

3.2.3.1 Moisture content

Moisture content was determined using the Chataway (1933) refractometric method. The refractive index of the honey samples was determined as described in section (3.2.2.1). The refractive index readings were converted to moisture content values using Chataway Table, which relates the refractive index of honey to its moisture content.

3.2.3.2 Free Acidity

The acidity of honey is the content of all free acids, expressed in milliequivalents / kg honey. The acidity was determined as described by Pearson (1976).

Ten grams of the triplicate samples were weighed and mixed with about 75 ml distilled water. Samples were titrated against (0.1 N) sodium hydroxide solution using phenolphthalein indicator the end point was determined by pink colour that persisted for seconds. The results, expressed as meq/kg honey, were calculated as follows :

$$\text{Free acidity} = V \times 10$$

Where:

V = Titer value " the volume of ml (0.1 N) NaOH used in the neutralization of 10 g honey".

3.2.3.3 Ash content

The ash content was determined according to A.O.A.C (1984) using muffle furnace. Two grams of honey sample were weighed accurately in to a pre-weighed dish, and gently heated in a muffle furnace until the samples became black and dry. The samples were ignited at 550°C to constant weight. The ash was determined by calculation and expressed as percentage using the Equation:

$$\text{Ash content} = \frac{W_1 - W_2}{W_0} \times 100$$

Where:

W_0 = Weight of honey taken.

W_1 = Weight of dish + ash.

W_2 = Weight of dish.

3.2.3.4 Crude protein

Crude protein was determined as total nitrogen by the micro-kjeldahl method as described by Pearson (1976), in the manner described below:

a) Digestion:

A 0.2 g of the triplicate samples were placed in the kjeldahl flask A 0.4g catalyst (potassium sulphate + copper sulphate) and 3.5 ml of concentrated sulphuric acid and were added mixed by gently swirling. The flask was placed on the heater until the liquid has become completely clean and of light blue green colour, sample was left to cool down to 40°C.

b) Steam distillation:

Ten millilitres of the boric acid solution (0.2 N) were added to a 100ml conical flask, and the flask was placed under the condenser of the distillation apparatus so that the outlet of the adapter dips in to the liquid. The content of kjeldahl flask was transferred to the distillation flask, and 15 ml of sodium hydroxide solution (40 %) were added and the liquid was kept boiling for 10 minutes. The nitrogen was collected in the conical flask containing boric acid and few drops of methyl red indicator. About 50 ml of distillation were collected after that heating was stopped.

c) Titration:

The content of the conical flask was titrated against hydrochloric acid (0.02 N) Solution. The volume was recorded.

The following formula was used to determine nitrogen percentage (g/100g):

$$\text{Nitrogen \%} = \frac{V \times N \times 14 \times 100}{W \times 1000}$$

Where :

V = Volume of hydrochloric acid used for titration.

N = Normality of hydrochloric acid (0.02 N).

W =Weight of original sample (g).

The nitrogen percentage was then multiplied by a factor (6.38) to determine the percentage of protein in the sample.

3.2.3.5 Reducing sugars

The reducing sugars (invert sugars)were determined by the method of Lane and Eynon (1923).

Two grams of honey were transferred to a flask and diluted with distilled water to 250 ml. A burette was rinsed with a portion of the honey solution and then filled up. Ten millilitres of fehling's solution were pipetted into a 250 ml erlenmeyer flask, a suitable amount of sample was run in this flask (about 15 ml of honey solution), and heated in a hot plate till boiling and continued for exactly two minutes. Five drops of methylene blue were added, then the titration was completed till the red colour appeared.

The reducing sugars were calculated from following formula :

$$\text{Reducing sugars \%} = \frac{\text{mg of reducing sugar/ 100ml} \times 250 \times 100}{\text{Weight of sample (mg)}}$$

Where:

mg of reducing sugars obtained from standard table according to the volume of titration.

3.2.3.6 Total sugars

The total sugars were determined according to Walker (1917) inversion method as follows :

a) Preparation of test sample:

Two grams of honey were transferred into 250 ml volumetric flask and distilled water was added till the mark.

b) Hydrolysis of test sample:

Fifty millilitres of test sample "honey solution " were pipetted into flask and heated to 65°C in a water bath, 10 ml of 6.34 N hydrochloric acid were added and kept for 3 minutes, then cooled to room temperature and neutralized with 5 M sodium hydroxide, using litmus paper as indicator and cooled again to 20°C. Then made to 100 ml with distilled water.

c) Titration:

The burette was rinsed and filled with the honey solution (after hydrolysis). Ten millilitres of Fehling's solution were pipetted into the erlenmeyer flask and 15 ml of the honey solution were added and heated on hot plate till boiling. Five drops of methylene blue were added and the titration was followed till the blue colour disappeared.

Total sugars were determined in the same way as reducing sugars (see section 3.2.3.5).

3.2.3.7 Glucose "Dextrose"

Glucose was determined according to Pearson (1976). Glucose is estimated iodometrically, reducing sugars by a copper reduction method and the fructose obtained by difference.

Two grams of honey sample were weighed and diluted with distilled water to 250 ml "honey solution ". Twenty five millilitres of this solution were pipetted into conical flask. A volume of 0.1 N iodine solution (at least twice that required for the reaction) and 100 ml sodium bicarbonate / carbonate solution were added. The flask was

placed in the dark for 2 hours, then acidified with 12 ml of 25 % sulphuric acid, and titrated with 0.1 N sodium thiosulphate. A blank was carried out at the same time and the difference between two titrations represent the glucose.

1 ml 0.1 N iodine = 0.009005 g (glucose).

3.2.3.8 Fructose

The fructose in honey sample obtained after determined dextrose by iodometrically method, and reducing sugars by copper reduction method (Pearson, 1976).

$$\text{Fructose \%} = \text{Reducing sugars \%} - \text{Dextrose \%}$$

3.2.3.9 Sucrose

The following formula is used to determine the apparent sucrose in honey sample, and the results are expressed as g apparent sucrose/100g honey (Walker, 1917).

$$\text{Apparent sucrose content \%} = (\text{invert sugar after inversion} - \text{invert sugar before inversion}) \times 0.95$$

3.2.3.10 Ascorbic acid content

The determination of ascorbic acid "vitamin C" was carried out according to A.O.A.C (1984) method.

Thirty grams of honey were blended with suitable amount of oxalic acid (0.4%) then made up to 250 ml with 0.4 % oxalic acid. Twenty

millilitres of this solution were pipetted to a beaker, then titrated with 2,6 dichlorophenol indophenol until faint pink colour appeared.

- Determination of dye strength

5 ml of standard ascorbic acid were added to 5 ml of 10 % oxalic acid solution in beaker and titrated with dye solution to faint pink colour.

$$\text{Dye strength} = \frac{1}{\text{Titer volume}}$$

Calculation

$$\text{Ascorbic acid mg /100g} = \frac{\text{Titer volume} \times \text{strength of dye} \times 100}{\text{weight of sample (mg)} \times \text{volume taken}}$$

3.2.3.11 Total Polyphenols

Total polyphenols were determined by prussion blue spectrophotometric method (Price and Butler, 1977).

Sixty milligrams of sample were shaken manually for a minute with 3 ml of methanol in a test tube. The mixture was filtered, then the tube was quickly rinsed with additional 3 ml of methanol and the contents poured at once into the funnel. The filtrate was mixed with 50 ml of water and analyzed within an hour. Three millilitres of 0.1 M FeCl_3 in 0.1 N HCl were added to 1 ml of filtrate, followed immediately by timed addition of 3 ml of 0.008 M $\text{K}_3\text{Fe}(\text{CN})_6$. The absorbance was read on UV /Vis spectrophotometer at 720 nm after 10 min. Tannic acid was used to make the standard curve following the same steps in the procedure.

The polyphenol content was calculated as follow:

$$\text{Polyphenol \%} = \frac{C \times 56 \times 100}{60}$$

Where:

C = Concentration corresponding to optical density.

56 = Volume of extract.

60 = Weight of sample in mg.

3.2.3.12 Minerals

Mineral extraction

Five grams of honey sample were weighed in dry crucible; the crucible was placed in muffle furnace at 550°C for 2 hours. The contents were cooled and transferred to 250 ml beaker, and 10 ml of 5 N HCl were added. The beaker was placed in a sand bath to boil for 10 min, and then 50 ml of distilled water were added and the content were filtered through Whatman's ashless filter paper No. 41, and the volume was made to 100 ml with distilled water. The extract was stored in bottles for mineral analysis (Chapman and Pratt, 1982).

From this extract the elements: Calcium, copper, iron, magnesium, manganese and zinc were determined using Perkin Elmer Atomic Absorption Spectrophotometer model No. 3110.

Sodium and Potassium

Sodium and potassium were determined according to the A.O.A.C (1984) using E.E.L. Flamephotometer. One millilitre of the extract was taken and diluted to 50 ml with distilled water. A standard NaCl solution was prepared by dissolving 3.33 g of NaCl powder in 1.0

litre of distilled water. Ten millilitres of solution were diluted to 1000 ml with distilled water giving 10 mg/l concentration. Standard solution of KCl was prepared by dissolving 2.54 g of KCl powder in 1.0 litre distilled water, then 10 ml of solution were taken and diluted to 1000ml with distilled water to give 10 mg/l concentration. The Flamephotometer was adjusted to zero using distilled water, and to 100 transmission using the prepared standard solution (NaCl and KCl), then the sample reading was recorded and the percentage of minerals was calculated as follows:

$$\text{Mineral} = \frac{\text{FR} \times \text{DF} \times 100}{\text{S} \times 1000 \times 1000}$$

Where:

FR = Flame photometer reading .

DF = Dilution factor.

S = Sample weight.

Phosphorous

Phosphorous determination was carried out according to Vanado molybdate method (A.O.A.C, 1984).

Five millilitres of sample were pipetted from the extract into 100 ml volumetric flask and 25 ml distilled water were added, within 5 min. Twenty millilitres of vanad– molybdate reagent were added, diluted to volume (100 ml) with distilled water, mixed and left to stand for 10 min, and then determination at 400 nm using Jenway 6305 UV /Vis spectrophotometer. Phosphorous concentration was determined from standard curve.

3.2.4 Statistical Analysis

All analyses were performed in triplicate. The analysis of variance (ANOVA) was performed to examine the significant effect in all parameters measured. least significant difference (LSD) test was used to separate between the means. The level of significance was $P \leq 0.05$ (Gomez and Gomez, 1984).

CHAPTER FOUR RESULTS AND DISCUSSION

4.1 Physical analysis

The refractive indices and pH values of four honey types were determined and their results were shown in figure 4.1 and 4.2 respectively.

4.1.1 Refractive index

The refractive index of four types of honey ranged between 1.4923 – 1.5038 (Figure 4.1). These results were similar to the findings of Lazaridou *et al.* (2004) who obtained 1.4892 – 1.5043 in selected Greek honeys and Ouchemoukh *et al.* (2007) who obtained 1.4889 – 1.4999 in some Algerian honeys.

4.1.2 pH

The pH of honey collected from the four floral sources was determined and the results were shown in Figure 4.2. The highest pH was found for sidir honey (5.00), while talih honey had the lowest pH (4.46). Significant differences ($p \leq 0.05$) were observed between the four honey samples. The pH range obtained for honeys from the different floral origin were found to be within the range of 3.42 – 6.10 reported by White *et al.* (1962), but slightly higher than the ranges 3.49 – 4.43, 3.56 – 4.79, 3.72 – 4.64 and 3.75 – 4.61 reported by Ouchemoukh *et al.* (2007), Terrab *et al.* (2004), Serrano *et al.* (2004) and Downey *et al.* (2005) for honey from different sources respectively.

4.2 Chemical analysis

4.2.1 Proximate composition

4.2.1.1 Moisture content

The moisture content of the honeys studied ranged from 13.2 to 17.6%. Sunflower honey had the highest moisture content, while talih honey had the lowest (Fig 4.3). These values are fairly agreed with

Fig 4.1: Refractive index of four honey types

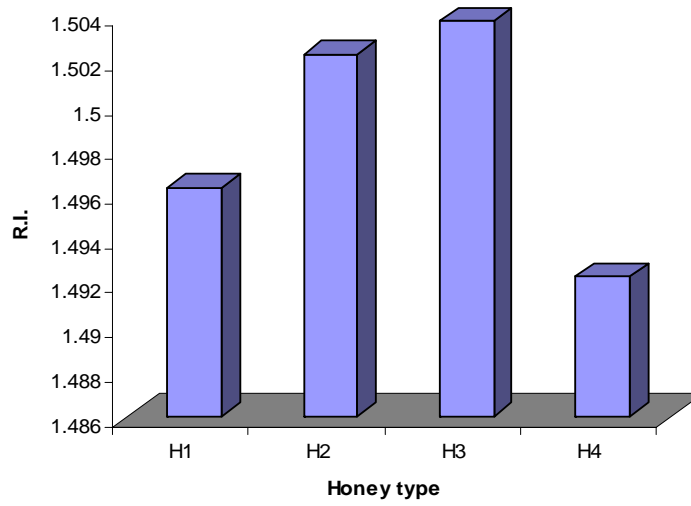
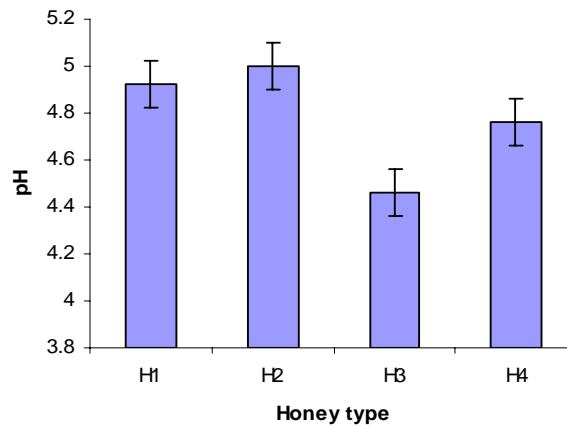


Fig 4.2 : pH of four honey types



H1 : sunut honey .
H2 : sidir honey.
H3 : talih honey.
H4 : sunflower honey.

the values of 13.1 – 19.9% obtained by Ibrahim (1985) and 13.0 – 18.9% given by Lazaridou *et al.* (2004). Other workers got slightly higher values, among them are White *et al.* (1962) 13.4 – 22.9%; Ouchemoukh *et al.* (2007) 14.64 – 19.04%; Serrano *et al.* (2004) 14.9 – 18.6%; Downey *et al.* (2005) 15.6 – 20.6% and Finola *et al.* (2007) 16.0 – 23.4%. These variations in the moisture content of honey under study could may be attributed to floral source, temperature, relative humidity, method of honey extraction and storage conditions.

4.2.1.2 Ash content

The honey, which contained the highest amount of ash was sunut honey (Figure 4.4), this may be due to high mineral content in sunut nectar (Ibrahim, 1985). No significant differences were observed between three types of honey (sidir, talih and sunflower), but significant differences were seen between sunut and other types of honey ($p \leq 0.05$). Ash content of Sudanese honey samples were found to be within the range reported in literature, e.g White *et al.* (1962); Ouchemoukh *et al.* (2007) and Terrab *et al.* (2004).

4.2.1.3 Protein content

Sunflower honey was found to contain the highest protein content 1.25%, where as sidir honey had the least 0.74 % (Figure 4.5). Statistical analysis gave significant differences between the samples except the differences between sidir and talih were non – significant ($p \leq 0.05$). Values obtained were found higher than the range 0.23 – 0.90 % reported by Ibrahim (1985) and the range of 0.25 – 0.76 % obtained by Schuette and Templin (1930). The increase in protein content in the honey samples, in the present study, indicate that it was mixed with large amount of pollen grains.

Fig 4.3 : Moisture content (%) of four honey types

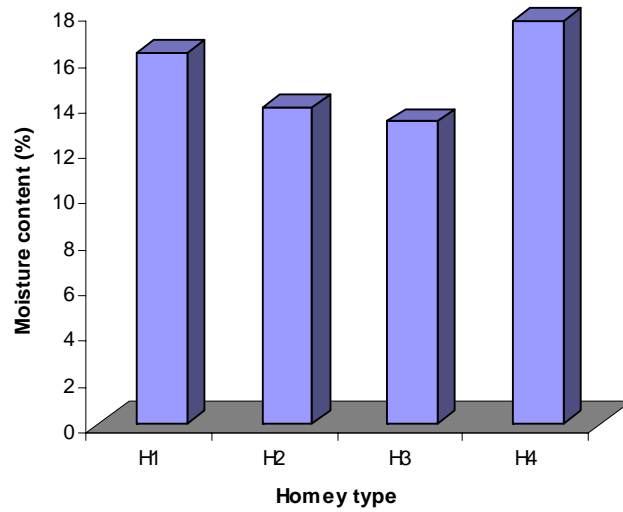
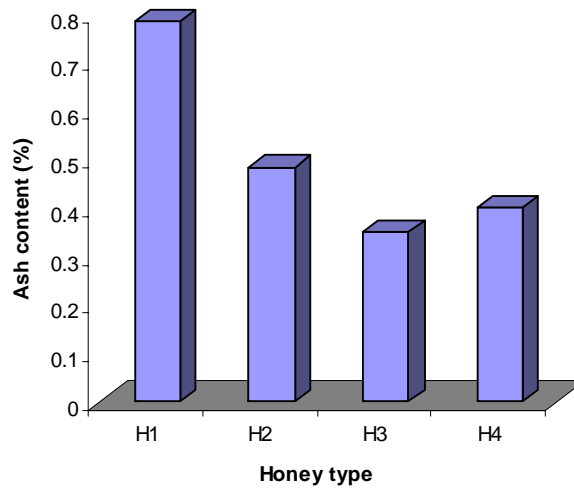


Fig 4.4 : Ash content (%) of four honey types



H1 : sunut honey.
H2 : sidir honey.
H3 : talih honey.
H4 : sunflower honey.

4.2.2 Carbohydrate content

4.2.2.1 Total sugar content

The percentages of the total sugars content in the four samples investigated were shown in Figure 4.6. Total sugars represent the largest portion of honey. Talih had the highest sugar content (81.5%) while sunut had the lowest (76.1%). Significant differences ($p \leq 0.05$) were observed between honey types. The values obtained in the present study were located within the range of 71.25 – 84.25% reported by Ouchemoukh *et al.* (2007), and were lower than the values obtained by Serrano *et al.* (2004) who reported 80.0 – 83.5%, but were higher than the range of 60.6 – 79.4% given by Ibrahim (1985).

4.2.2.2 Reducing sugar content

From Figure 4.7 it can be seen that the reducing sugars in honey samples fall in the range of 72.6 – 79.3%. The results show significant differences in reducing sugars of honey samples ($p \leq 0.05$). These results are compatible with the findings of Ouchemoukh *et al.* (2007) who reported a range of 67.83 – 80.25%, but higher than the range 57.8 – 75.7% given by Ibrahim (1985).

4.2.2.3 Glucose content

The glucose content of honeys under investigation ranged from 25.9% (sidir) to 40.5% (talih) as shown in Figure 4.8. The values obtained show significant differences between all honey samples except between sunflower and sunut where insignificant differences were observed ($p \leq 0.05$). These values are lower than the range of 29.4 – 42.0% reported by Ouchemoukh *et al.* (2007), but higher than the ranges 13.5 – 36.3% and 24.0 – 39.7% obtained by Lazaridou *et al.* (2004) and Finola *et al.* (2007). The observation as well as the data presented in this study for glucose content fully agreed with the range 22.0 – 40.7 % given by White *et al.* (1962).

Fig 4.5 : protein content (%) of four honey types

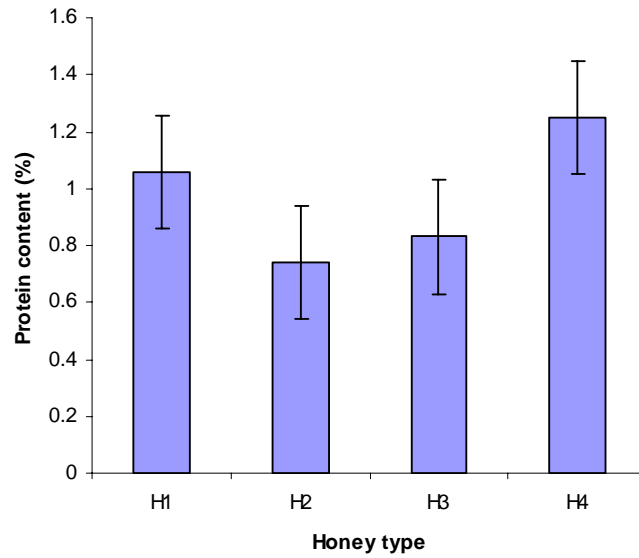
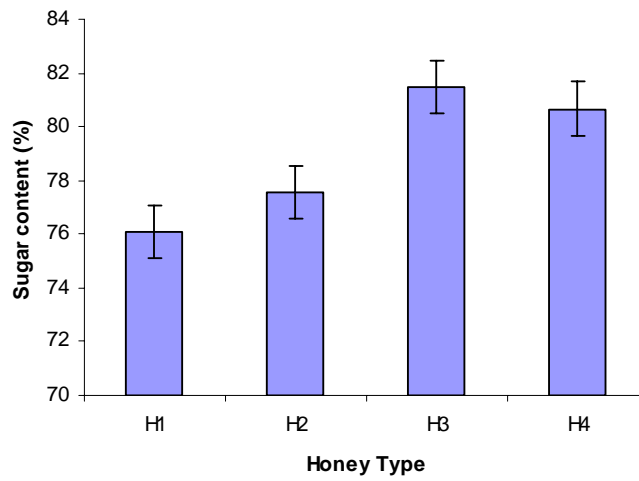


Fig 4.6 : Total sugar content (%) of four honey types



H1 : sunut honey .
H2 : sidir honey.
H3 : talih honey.
H4 : sunflower honey.

4.2.2.4 Fructose content

Figure (4.9) exhibits the fructose content of the honey samples, which varied from 37.2% (sunut) to 47.9% (sidir). Insignificant differences were observed between three types of honey (sunut, talih and sunflower) but significant differences were seen between sidir and other types of honey ($p \leq 0.05$). These values fall within the range of 33.0 – 48.4 % obtained by Finola *et al.* (2007), but higher than the range 27.2 – 44.3 % reported by White *et al.* (1962) and the range 22.1 - 41.3 % reported by Lazaridou *et al.* (2004).

4.2.2.5 Sucrose content

Sucrose content in the honey samples are presented in Figure 4.10. The level of sucrose in Sudanese honey from different floral source ranged from 2.1 % (talih) to 3.6 % (sidir). Statistical analysis show insignificant differences between honey samples ($p \leq 0.05$). These values were located within the range 0.2 – 7.6 % given by White *et al.* (1962); 0.08 – 5.31 % reported by Ouchemoukh *et al.* (2007) and the range 0.14 – 11.49 % recorded by Serrano *et al.* (2004). The amount of sucrose in honey varied according to the floral source and invertase enzyme activity. Maximum sucrose content of 5 % is required by the food regulations of many countries and proposed Codex Alimentarius (1969).

4.2.3 Ascorbic acid content (vitamin C)

Figure 4.11 shows that sunut and talih honeys had the highest level of ascorbic acid 14.9 and 11.3 mg / 100gm respectively, while sunflower honey had the lowest content (7.8 mg / 100gm). Significant differences ($p \leq 0.05$) were observed between honey types. Values

Fig 4.7 : Reducing sugar content (%) of four honey types

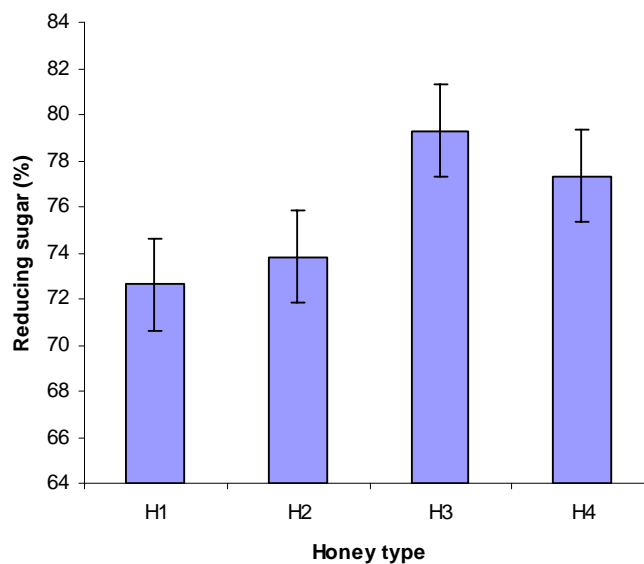
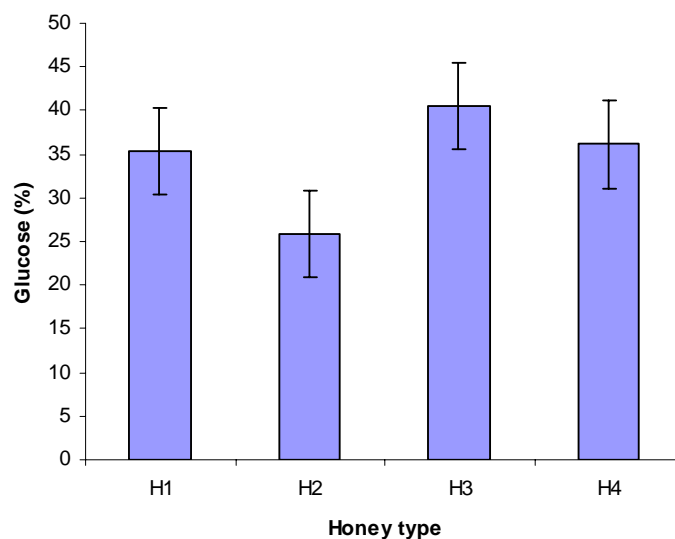


Fig 4.8 : Glucose content (%) of four honey types



H1 : sunut honey .
H2 : sidir honey.
H3 : talih honey.
H4 : sunflower honey.

obtained fairly agreed with the values recorded by Griebel (1938) 7 – 22 mg / 100 gm, and slightly higher than range of 1.1 – 14.6 mg / 100gm reported by Werder and Antener (1938). The increase in vitamin C content in the honey samples could be due to the floral source and /or honey was mixed with large amount of pollen grains.

4.2.4 Total polyphenols

Total polyphenol values in the four samples studied were shown in Figure 4.12 as 83.7, 119.8, 120.8 and 121.4 mg / 100gm for sunflower, sidir, talih and sunut respectively. Insignificant differences were observed between the honey types with the exception of H4 sample (sunflower) which was shown to be significantly different from other samples ($p \leq 0.05$). Values obtained were within the range given by Ouchemoukh *et al.* (2007) who reported 64.0 – 130.4 mg/ 100gm. The values obtained were higher than the range reported by several workers such as Yaoa *et al.* (2004), Yaoa *et al.* (2005) and Meda *et al.* (2005) who reported ranges of 2.14 – 10.3; 2.13 – 12.11 and 32.59 – 114.75 mg/ 100gm respectively. The amount of polyphenols in honey samples depends on the floral origin.

4.2.5 Free acidity

Figure 4.13 shows great variations in the acidity of the honey samples which ranged between 37.0 – 63.7 meq/kg. Sunut had the highest free acidity, while sidir had the lowest value. The results recorded significant differences between the samples ($p \leq 0.05$). Values obtained were lower than the range of 6 – 171 meq/kg reported by Ibrahim (1985), and higher than the values obtained by Finola *et al.* (2007), Terrab *et al.* (2004), Costa *et al.* (1999) and Downey *et al.*

Fig 4.9 : Fructose content (%) of four honey types

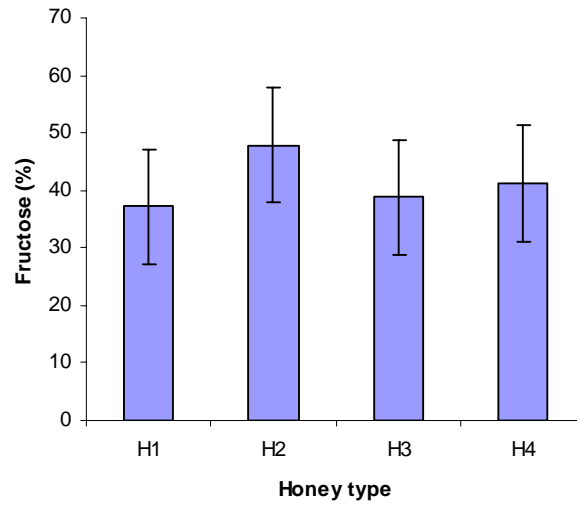
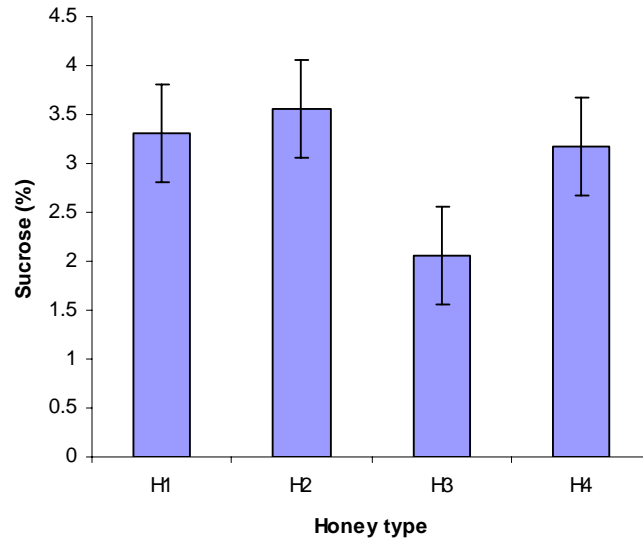


Fig 4.10 : Sucrose content (%) of four honey types



H1 : sunut honey .
H2 : sidir honey.
H3 : talih honey.
H4 : sunflower honey

(2005) who reported 11.9 – 29.4, 17.59 – 39.81, 24.4 – 53.3 and 17.1 – 50.9 meq/kg respectively. Free acidity in the honey samples depends on the floral origin and storage condition.

4.2.6 Minerals content

The mineral content in the four types of Sudanese honey was determined and the results were presented in Table 4.1.

4.2.6.1 Potassium content

The predominating mineral element in honey is potassium (White and Rudyj, 1978). The honeys studied varied greatly in their potassium content (Table 4.1) sunut had the highest amount (3318.25 ppm). These values were higher than 639 – 1845 ppm given by Ankalm (1998) and 261 – 1380 ppm reported by Terrab *et al.* (2004).

4.2.6.2 Sodium content

Sodium content of honey types studied ranged from 438.9 ppm (talih) to 858.7 ppm (sunut). Significant differences were observed between honey samples ($p \leq 0.05$). Values obtained were higher than 256 – 501 ppm reported by Terrab *et al.* (2004), 674 mg/kg given by Hernandez *et al.* (2005) and 354 $\mu\text{g/g}$ reported by Özcan *et al.* (2006).

4.2.6.3 Phosphorus content

Phosphorus content in honey samples, under study, ranged from 70.8 ppm (sidir) to 102.7 ppm (sunut). Statistical analysis show significant differences between honey samples ($p \leq 0.05$). Values obtained fall within the range of 63.8 – 143 ppm given by Ankalm (1998), and lower than 436 $\mu\text{g/g}$ reported by Özcan *et al.* (2006), but higher than 26 – 96 ppm given by Terrab *et al.* (2004).

Fig 4.11: Vitamin C content (mg/100gm) of four honey types

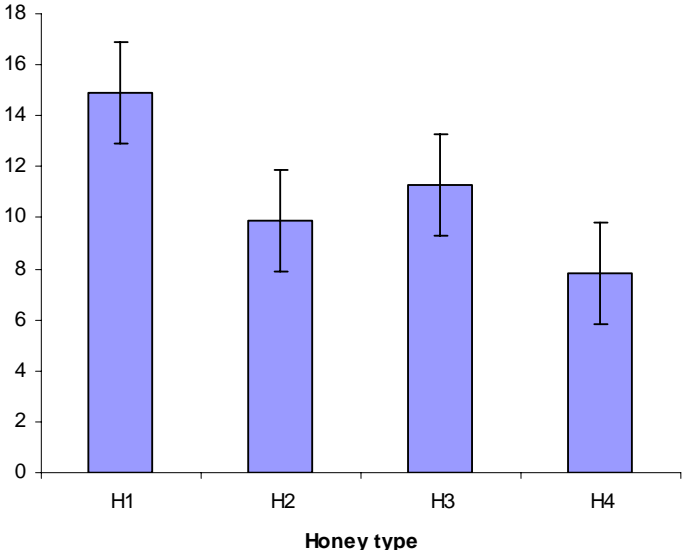
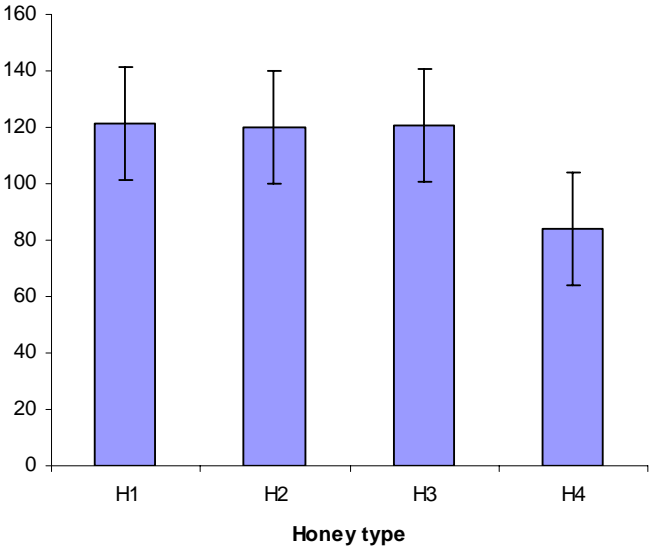


Fig 4.12: Total polyphenol content (mg/100gm) of four honey types



H1 : sunut honey .
H2 : sidir honey.
H3 : talih honey.
H4 : sunflower honey.

4.2.6.4 Iron content

Iron content of the honey types was shown in Table (4.1). Sunut was found to contain the highest iron content (50.2 ppm), while talih had the least (39.8 ppm). Insignificant differences were observed between honey types with the exception of H₁ sample (sunut) which was shown to be significantly different from other samples ($p \leq 0.05$). The results obtained in the present study were higher than the results found by Hernandez *et al.* (2005) who reported 4.69 mg / kg, but lower than those of Özcan *et al.* (2006) who reported 80.3 µg / g.

4.2.6.5 Magnesium content

Magnesium level of honeys studied ranged from 17.5 ppm (talih) to 37.7 ppm (sunut). Significant differences were observed between honey samples ($p \leq 0.05$). Values obtained were lower than 37 – 139 ppm reported by Terrab *et al.* (2004) and 212 µg/g given by Özcan *et al.* (2006). However the results agreed with the value 33.9 mg/kg obtained by Hernandez *et al.* (2005).

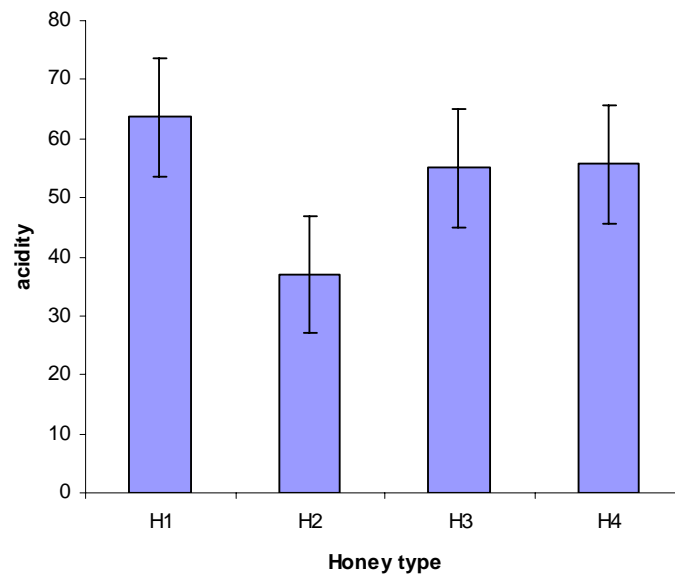
4.2.6.6 Calcium content

The level of calcium in honeys ranged from 9.6 ppm (sunut) to 24.5 ppm (sidir). Significant differences were observed between honey samples ($p \leq 0.05$). The results were lower than 111 – 257 ppm reported by Ankalm (1998), and 110 – 248 ppm given by Terrab *et al.* (2004).

4.2.6.7 Manganese content

Manganese content of honey samples ranged from 1.95 ppm (sunut) to 11.70 ppm (talih). Significant differences were observed between

Fig 4.13: Free acidity (meq/kg) of four honey types



H1 : sunut honey .
H2 : sidir honey.
H3 : talih honey.
H4 : sunflower honey.

honey samples ($p \leq 0.05$). Values obtained were lower than 33.9 mg/kg reported by Hernandez *et al.* (2005), but higher than 0.84 $\mu\text{g/g}$ given by Özcan *et al.* (2006).

4.2.6.8 Copper content

Copper content ranged from 3.80 ppm (sidir) to 5.55 ppm (sunflower). Statistical analysis shows insignificant difference between all honey samples ($p \leq 0.05$). These values were higher than 0.33 mg/kg reported by Hernandez *et al.* (2005).

4.2.6.9 Zinc content

Zinc level of honeys ranged from 2.7 ppm (talih) to 4.01 ppm (sunut). Insignificant differences were observed between honey types with the exception of H₁ sample (sunut) which was shown to be significantly different from other samples ($p \leq 0.05$).

4.3 Pollen grains Analysis

Each species of plant has characteristics pollen type. Thus, the pollen grains from most species can be distinguished by their outer form and / or by their chemical composition or content of nutrients.

In this study, trials are made to identify kinds and shapes of pollen grains present in each honey sample, from the results obtained the pollen grains content in each honey sample:

H1: pollen grain of *Acacia nilotica*

H2: pollen grain of *Zizyphus spina-christi*

H3: pollen grain of *Acacia seyal*

H4: pollen grain of *Helianthus annuus*

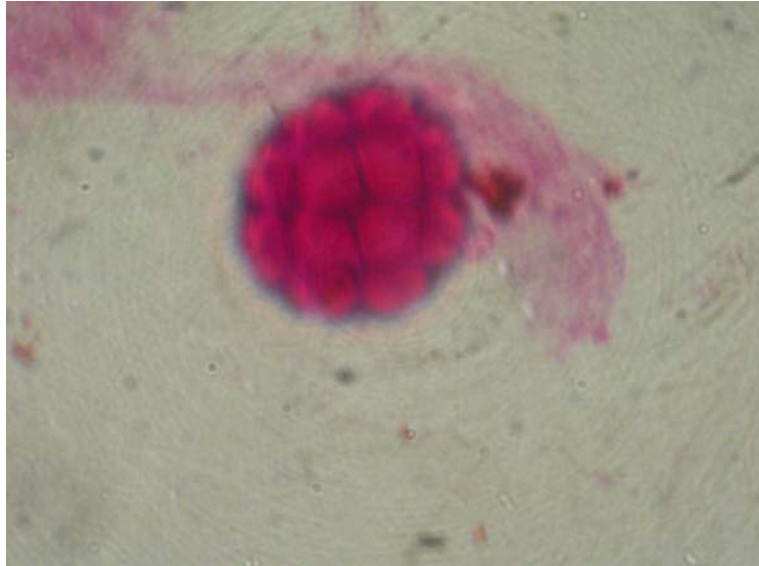
Table 4.1 : Mineral content (ppm) in four types of Sudanese honey

Minerals	Types of honey			
	H1	H2	H3	H4
K	3318.25 ^a (±186.6)	2297.75 ^c (±213.9)	1774.50 ^d (±248.8)	2697.50 ^b (±28.15)
Na	858.70 ^a (±104.3)	582.70 ^{bc} (±91.7)	438.90 ^c (±66.1)	655.50 ^b (±32.01)
P	102.70 ^a (±1.3)	70.80 ^c (±3.74)	84.90 ^b (±11.5)	72.20 ^c (±5.39)
Fe	50.20 ^a (±2.7)	39.90 ^b (±3.18)	39.80 ^b (±1.3)	41.77 ^b (±3.56)
Mg	37.70 ^a (±1.3)	25.10 ^c (±1.22)	17.50 ^d (±1.25)	30.30 ^b (±2.57)
Ca	9.60 ^c (±0.6)	24.50 ^a (±2.53)	18.40 ^b (±3.18)	19.70 ^b (±2.1)
Cu	5.11 ^a (±0.25)	3.76 ^a (±1.21)	5.00 ^a (±1.7)	5.55 ^a (±0.59)
Mn	1.95 ^b (±0.31)	2.01 ^b (±1.38)	11.70 ^a (±4.26)	11.37 ^a (±5.56)
Zn	4.01 ^a (±0.56)	3.13 ^{ab} (±0.83)	2.68 ^b (±0.38)	3.10 ^{ab} (±0.59)

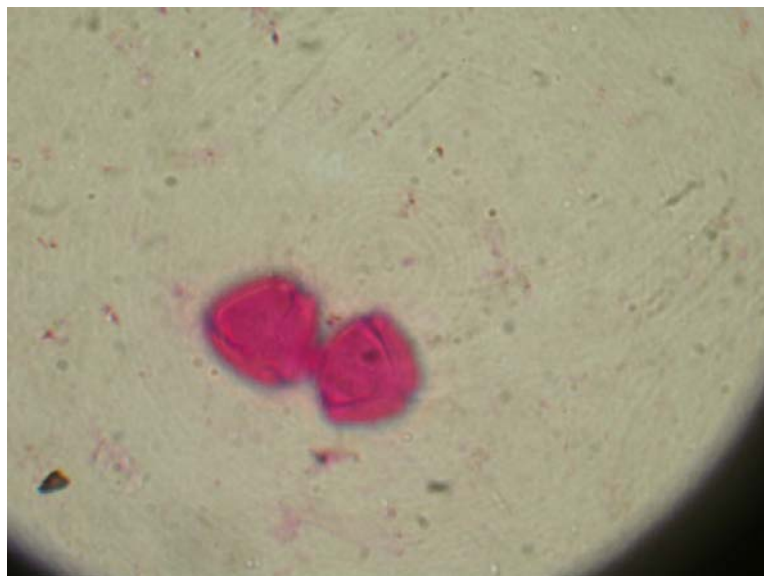
Values are means (± SD). Means not sharing a common superscript letter in a column are significantly different at $p \leq 0.05$ assessed by Duncan's multiple range

H1 : sunut honey, H2 : sidir honey, H3 : talih honey, H4 : sunflower honey.

The results of pollen grains analysis of four honey samples from different floral origin



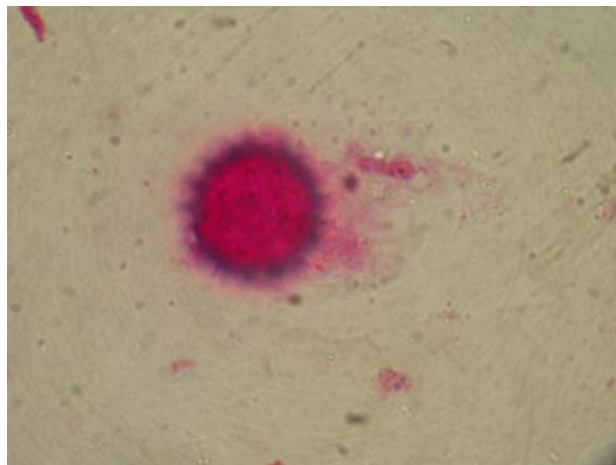
Sunut pollen grain (H_1)



Sidir pollen grain (H_2)



Talih pollen grain (H₃)



Sunflower pollen grain (H₄)

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

From The results and findings of the present investigation, it can be concluded that:

- 1- Honey is a valuable food since it contains so many nutrients.
- 2- There is a significant variation between different honey types according to floral origin.
- 3- Sunt honey was observed to contain the highest level of ash, ascorbic acid, total polyphenols, acidity, potassium, sodium, phosphorus, iron magnesium and zinc compared to other types of honey.
- 4- The result also revealed that sider honey had the highest amount of sucrose, fructose and calcium.
- 5- Talh honey had been highest in total sugars and manganese.
- 6- For proximate composition and mineral content, sun flower honey had the highest level of moisture, protein and copper content.
- 7- This study demonstrated that honey collected from different floral origin significantly varied in nutritive value.

Recommendations

- A standard characteristic guide lines of Sudanese honey must be established so as to classify the sort of honey.
- Bee honey is considered to be valuable nutrients as a source of sugars and wide range of different constituents such as minerals, vitamins, enzymes and others, then it is recommended that human diet must consists of some honey.
- Further studies are needed in order to know the direct effect of Pollen and floral origin on the nutritive value of honey.

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APPENDIX

Table (1) : Physical Properties of four honey types

Types of Honey	(R.I.) at corrected temperature (20⁰C)	PH
H₁	1.4963	4.92 ^b (± 0.015)
H₂	1.5023	5.00 ^a (± 0.01)
H₃	1.5038	4.46 ^d (± 0.0006)
H₄	1.4923	4.76 ^c (± 0.03)

Values are means (± SD). Means not sharing a common superscript letter in a column are significantly different at $p \leq 0.05$ assessed by Duncan's multiple range

H1 : sunut honey .

H2 : sidir honey.

H3 : talih honey.

H4 : sunflower honey.

Table (2): Chemical composition of four honey types

Parameter	Types of Honey			
	H1	H2	H3	H4
Moisture Content (%)	16.2	13.8	13.2	17.6
Ash Content (%)	0.78 ^a (± 0.1)	0.48 ^b (± 0.08)	0.35 ^b (± 0.09)	0.40 ^b (± 0.1)
Protein Content (%)	1.06 ^b (± 0.2)	0.74 ^c (± 0.05)	0.83 ^c (± 0.05)	1.25 ^a (± 0.1)
Free Acidity (meq/kg)	63.70 ^a (± 2.1)	37.00 ^c (± 1.7)	55.00 ^b (± 1)	55.70 ^b (± 1.2)
Vitamin C (mg/100g)	14.90 ^a (± 0.7)	9.90 ^c (± 0.7)	11.30 ^b (± 0.7)	7.80 ^d (± 0.7)
Polyphenols (mg/100mg)	121.40 ^a (± 0.4)	119.80 ^a (± 1.9)	120.80 ^a (± 0.4)	83.70 ^b (± 0.6)

Values are means (± SD). Means not sharing a common superscript letter in a column are significantly different at $p \leq 0.05$ assessed by Duncan's multiple range

H1 : sunut honey .
H2 : sidir honey.
H3 : talih honey.
H4 : sunflower honey.

Table (3) : Carbohydrate content in four honey types

Parameter	Types of Honey			
	H ₁	H ₂	H ₃	H ₄
Total Sugar (%)	76.1 ^c (± 2.2)	77.6 ^{bc} (± 1.3)	81.5 ^a (± 1.5)	80.7 ^{ab} (± 2.4)
Invert Sugar (%)	72.6 ^c (± 1.7)	73.8 ^{bc} (± 2.6)	79.3 ^a (± 2.3)	77.3 ^{ab} (± 1.1)
Glucose (%)	35.4 ^b (± 1.4)	25.9 ^c (± 1.1)	40.5 ^a (± 2.3)	36.1 ^b (± 2.3)
Fructose (%)	37.2 ^b (± 0.9)	47.9 ^a (± 2.1)	38.8 ^b (± 4.4)	41.2 ^b (± 2.5)
Sucrose (%)	3.3 ^a (± 0.5)	3.6 ^a (± 1.4)	2.1 ^a (± 0.8)	3.2 ^a (± 1.4)

Values are means (± SD). Means not sharing a common superscript letter in a column are significantly different at $p \leq 0.05$ assessed by Duncan's multiple range

H₁ : sunut honey.
H₂ : sidir honey.
H₃ : talih honey.
H₄ : sunflower honey.