بسم الله الرحمن الرحيم

Sudan University of Science andTechnology

Collage of Graduate Studies

Detection of the Identification Some Sudanese Bee Honey Samples

إختبار الغش لبعض عينات عسل النحل السوداني

A Thesis Submitted in Partial Fulfilmentof the Requirements for the M.Sc Degree in Plant Protection

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بسم الله الرحمن الرحيم

قال تعالى:

(وَأَوْحَى رَبُّكَ إِلَى النَّحْلِ أَنْ اتَّخِذِي مِنْ الْجِبَالِ بُيُوتاً وَمِنْ الشَّجَرِ وَمِمَّا يَعْرِشُونَ(68) ثُمَّ كُلِي مِنْ كُلِّ الثَّمَرَاتِ فَاسْلُكِي سُبُلَ رَبِّكِ ذُلُلاً يَخْرُجُ مِنْ بُطُونِهَا شَرَابٌ مُخْتَلِفٌ أَلْوَانُهُ فِيهِ شِفَاءٌ لِلنَّاسِ إِنَّ فِي ذَلِكَ لآيَةً لِقَوْم يَتَفَكَّرُون).

صدق الله العظيم

سورة النحل (68 -69)

DEDICATION

I dedicate this WORK to

My mother . . .

Who gives me a lot of care and support

To my father . . . .

Who always advises me in my academic journey

To my ***brothers*** *and* ***sisters*** *my stars in darkness*

To all Family in Darfur south S state *S and my dear*

sisters ***Mariam and Hawaa***

ACKNOWLEDGEMENT

*Firstly I thank **ALLAH** who give me the health and **Dr: Abdel***

Bagi El – Sayed Ali

Power to learn and write this work

I thank all people who support me in my academic

Especially those who help me to write this work

My teachers and laboratory technicians

Finally I found no word to express my respect to

who gave me a lot of thought and advices

I have to say thank you very much my great supervisor.

Ibrahim

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Abstract

Laboratory experiments Conductedat the Faculty ofAgricultural Studies, Sudan University of Science and Technology and the central lab at the University of Khartoum toStudy on detemination of adultaration some different samples of sudanese bee honey

, conducted a preliminary experiment to detect the moisture content in the samples honey and so for ways to save samples of segments different diet weights were treated with honey were weighed for a period of five days 'was the highest percentage in weight is (8.04) in the control after 48 hours of treatment it was clear.

In the same context, the chemical analysis laboratory procedure(JICA) College of Agricultural Studies to determine the moisture content has been taken (5g) of each sample to determine the moisture content and pH were taken (3g) to determine the percentage of purity was the highest percentage of moisture in both termed South Darfur and theRiver Nilestate by (8.33%) and the lowest percentage of moisture in the Omdurman market sample recorded by (3.66%) and the highest percentage of purity was in the sample of South Darfur (0.0025%) and the lowest percentage of purity in a sample of the River Nile state reaching purity where the ratio (% 0.965) and a higher percentage of acidity was in a sample of the RiverNile state and was by (0.125%) and the lowest rate in sample of Omdurman market was by (0.025%)

In the samesequence it has been part of the bee honey samples analysis Central Laboratory, of KhartoumUniversity - shambat to determine the ratio of sugars where they were taken (20mg / ml) from the solution and through the experience of get obstructed sugar maltose, sucrose, while rates appeared degrees in each of the sugar glucose and fructose in different proportions where it was percentagehighest sugar glucose in a sample Omdurman market (% 15.15) and the lowest in the sample of South Darfur (5.81%) and the Fructocehighest percentage were in the sample Omdurman market (26.45%) and the least in a sample of South Darfur was the rate of (9.26%).

اطروحة البحث

اجريت هذه التجارب المعملية بكلية الدراسات الزراعية ، جامعة السودان للعلوم والتكنولوجيا والمعمل المركزي بجامعة الخرطوم لكشف الغش في عسل النحل السوداني ، واجريت تجربة اولية لكشف نسبة الرطوبة في العينات العسلية وذلك عن طرق حفظ عينات من شرائح لحمية مختلفة الاوزان تمت معاملتها بالعسل وتم وزنها لفترة خمس ايام ٬ فكانت اعلى نسبة في الوزن هي(8.04) في الشاهد بعد 48 ساعة من المعاملة فكانت واضحة

وفي ذات السياق تم اجرا التحليل الكيميائي بمعمل جايكا بكلية الدراسات الزراعية لتحديد نسبة الرطوبة وتم اخذ (5g) من كل عينة لتحديد نسبة الرطوبة والحموضة وتم اخذ (3g) لتحديد نسبة النقاء فكانت اعلى نسبة للرطوبة في كل من عينتا جنوب دارفور وولاية نهر النيل بنسبة (8.33%) واقل نسبة رطوبة في عينة سوق امدرمان التي سجلت بنسبة (3.66%) و اعلى نسبة نقاء كانت في عينة جنوب دارفور (0.0025%) واقل نسبة نقاء في عينة ولاية نهرالنيل حيث بلغت نسبة النقاء فيها (0.965%) واعلى نسبة حموضة كانت في عينة ولاية نهر النيل وكانت بنسبة(0.125%) واقل نسبة في عينة سوق امدرمان وكانت بنسبة (0.025%)

وفي ذات الاطار تم تحليل جزء من العينات العسلية بالمعمل المركزي جامعة الخرطوم – شمبات لتحديد نسبة السكريات حيث تم اخذ (20mg / ml) من المحلول ومن خلال التجربة تعسر الحصول لسكر المالتوز والسكروز بينما ظهرت نسب متفاوتة في كل من سكر الجلكوز والفراكتوز بنسب مختلفة حيث كانت اعلى نسة لسكر الجلكوز في عينة سوق امدرمان (15.15%) واقلها في عينة جنوب دارفور (5.81%) واعلى نسبة للفراكتوز كانت في عينة سوق امدرمان (26.45%) واقلها في عينة جنوب دارفور وكانت بنسبة (9.26%).

CHAPTER ONE

Introduction

Honey is 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. The composition and properties of honey vary with the floral and honeydew sources utilized by honeybees, as well as regional and climatic conditions (Ahmed *et al*., 2007) .

The chemical composition of honey is including sugars, proteins moisture, vitamins, minerals, hydroxymethylfurfural (HMF), enzymes, flavonoids, phenolic acids, volatile compounds etc., However, the main constituents of honey are moisture, glucose, fructose, sucrose, minerals, and proteins (Gheldof, 2002).

Sudan, is the one of the largest country in Africa, with its different climatic conditions ranging from Sahara and sub-Sahara, savannah and tropical regions possesses a tremendous wealth of terrestrial plants which contribute to the economy of the country. Medicinal plants represent an important part of these resources with great potentialities and research in this field is encouraged by different institutions in public and private sectors. Sudanese floral honey in the last decades gained a solid ground and interestin the field of commerce and research, but the available honey products of different origins lack documentation in the literature about their composition and properties. The present research was undertaken to spot more light on the composition of floral honeys from different regions in Sudan with emphasis on their antioxidant polyphenols. While there are various types of antioxidants naturally occurring in honey as mentioned previously, this study focuses only on the flavonoids.(Ahmed, 2006)

World honey production is mainly located in Asia, Europe and North America. Africa and South America also has some production. Output in Oceania is lower relatively.

In Asia honey producing areas are mostly distributed in China, Turkey and India. The honey yield of these three countries accounts for more than 85% of Asia total output.United State of America, Mexico and

Canada are the main honey producing countries in North America, over 90% proportion of total output. In Europe, Ukraine, Russia, Spain, France, Germany, Hungary, Greece and Italy etc. all have some honey production every year. In Africa, honey production is mainly located in Ethiopia, Kenya, Tanzania, Angola and Egypt. Argentina and Brazil shares over 70% of honey yield in South America. In Oceania, Australia and New Zealand occupied more than 95% of Oceanian honey output.(Adebolu, 2005)

In addition to its reputation as Nature's nutritive sweetener, research also indicates that honey's unique composition makes it useful as an antimicrobial agent and antioxidant.

The health benefits of honey - like all foods - depend on the quality of the honey. But in this case, the situation is even more extreme, because the pollen that collects on the bees' legs as they move from plant to plant is only as healthful and as diverse as those plants.(Al-Waili , 2004)

In a series of experiments involving healthy subjects and those with either high cholesterol or type 2 diabetes, honey has proved itself the healthiest sweetener. For 15 days, 8 healthy subjects, 6 patients with high cholesterol, 5 patients with high cholesterol and high C-reactive protein (a risk factor for cardiovascular disease), and 7 patients with type 2 diabetes were given solutions containing comparable amounts of sugar, artificial honey or natural honey .

Researchers from the Penn State College of Medicineasked parents to give either honey, honey-flavored dextromethorphan (DM), or no treatment to the children. The first night, the children did not receive any treatment. The following night they received a single dose of buckwheat honey, honey-flavored DM, or no treatment 30 minutes before bedtime. The trial was partially blind as parents could not distinguish between the honey and the medication, although those administering no medication were obviously aware of the fact.(Paul , 2007)

Honey boosts immunity. Research conducted in several hospitals in Israel found honey effective in decreasing the incidence of acute febrile neutropenia(when high fever reduces white blood cell count) in64% of patients.

Honey also reduced the need for Colony Stimulating Factor(a compound produced in the cells lining the blood vessels that stimulate bone marrow to produce more white blood cells) in 60% of patients with acute febrile neutropenia; increased neutrophil count (another type of white blood cell), decreased thrombocytopenia (low platelet count), and stabilized hemoglobin levels at>11 gm/dl (a bit low but way better than full blownanemic).

In a preliminary announcement at the 2nd International Conference on the Medicinal Use of Honey in 2010 there is a preliminary announcement that intracervical injection of honey in women with chronic endocervitis was of positive therapeutic value bothin terms of clinical cure and fertility enhancement At the same conference it was reported that honey has positive effect on the mechanical properties of thefetal membranes, may be through "collagen promoting action.(salehi,2014)

Honey adulteration is a complex problem, which has a significant economic impact; it can be occurred by the addition of different materials. Adulteration, or the addition of foreign substances to honey such as; molasses, starch solution, glucose, sucrose, water and inverted sugar, were studied by Physiochemical characteristic of Egyptian honey were studied by adulteration usually refers to mixing other matter (substance) of an inferior and sometimes harmful quality with food or drink intended to be sold. With companies concerned about the bottom line, the temptation to cheat is considerable, and unfortunately, the adulteration of honey is a serious economic and regulatory problem. As usual, the losers are the consumers and the processor or re-processor seeking to provide a wholesome product that meets regulatory standards. From an economic point of view, food product adulteration can destabilize the market by bringing in unfair competition(Bogdanov, 2004. Bogdanov, 2010).

Adulteration in Sudan there is alack of information on honey adulteration is sudan .(Mohammed, 2007) found the some samples of honey collection know the local markeds ware adultered.

This study was to investagation the adulteration of some sudanese bee honey samples collect from different states .

CHATER TWO

Literature Review

2:1 Bee honey

Honey is the natural sweet substance, produced by honeybees from the nectar of plants or from secretions of living parts of plants, or excretions of plant-sucking insects(Arcot, 2002) on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature.

2:1:1 Physical characteristics

2:1:1:1 Viscosity

Freshly extracted honey is a viscous liquid.(Alkhalifa, A.S. and Alrify, 1999).Its viscosity depends on a large variety of substances and therefore varies with its composition and particularly with its water content Viscosity is an important technical parameter during honey processing, because it reduces honey flow during extraction, pumping, settling, filtration, mixing and bottling. Raising the temperature of honey lowers its viscosity a phenomenon widely exploited during industrial honey processing. Some honeys, however, show different characteristics in regard to viscosity: Heather (Calluna vulgaris) Manuka (Leptospermum scoparium) and Carviacallosa are described as thixotrophic which means they are gel-like (extremely viscous) when standing still and turn liquid when agitated or stirred. By contrast a number of Eucalyptus honeys show the opposite characteristics. Their viscosity increases with agitation.

2:1:2 Colour

Colour in liquid honey varies from clear and colourless (like water) to dark amber or black The various honey colours are basically all nuances of yellow amber, like different dilutions or concentrations of caramelized sugar, which has been used traditionally as a colour standard More modern methods for measuring honey colour are described below. Colour varies with botanical origin, age and storage conditions, but transparency or clarity depends on the amount of suspended particles such as pollen. Less common honey colours are bright yellow (sunflower) reddish undertones (chestnut) greyish (eucalyptus) and greenish (honeydew). Once crystallized, honey turns lighter in colour because the glucose crystals are white. Some of the honeys reportedly "as white as milk" in some parts of East Africa are finely crystallized honeys which are almost water white, i.e. colourless, in their liquid state. (Binkley D, 2014)

The most important aspect of honey colour lies in its value for marketing and determination of its end use. Darker honeys are more often for industrial use, while lighter honeys are marketed for direct consumption. In many countries with a large honey market, consumer preferences are determined by the colour of honey (as an indication of a preferred flavour) and thus, next to general quality determinations, colour is the single most important factor determining import and wholesale prices.

Honey colour is frequently given in millimetres on a Pfund scale (an optical density reading generally used in international honey trade) or according to the U.S. Department of Agriculture classifications .

2:1:3 Crystallization

Crystallization is another important characteristic for honey marketing, though not for price determination. In temperate climates most honeys crystallize at normal storage temperatures this is due to the fact that honey is an oversaturated sugar solution, i.e. it contains more sugar than can remain in solution. Many consumers still think that if honey has crystallized it has gone bad or has been adulterated with sugar.

The crystallization results from the formation of monohydrate glucose crystals, which vary in number, shape, dimension and quality with the honey composition and storage conditions. The lower the water and the higher the glucose content of honey, the faster the crystallization. Temperature is important, since above 25 ° and below 5 °C virtually no crystallization occurs. Around 14°C is the optimum temperature for fast crystallization, but also the presence of solid particles (e.g. pollen grains) and slow stirring result in quicker crystallization Usually, slow crystallization produces bigger and more irregular crystals.

During crystallization water is freed. Consequently, the water content of the liquid phase increases and with it the risk of fermentation. Thus, partially crystallized honey may present preservation problems, which is why controlled and complete crystallization is often induced deliberately. In addition, partially crystallized or reliquified honey is not an attractive presentation for retail shelves (Kantor, 1999).

2:2:1 **Honey composition**

The average composition of American honeys, more or less representative of all honeys, is shown in lists the various components identified in honeys from all around the world.

2:2:2 Sugars

Sugars account for 95to 99% of honey dry matter. The majority of these are the simple sugars fructose and glucose which represent *85-95%* of total sugars. Generally, fructose is more abundant than glucose This predominance of simple sugars and particularly the high percentage of fructose are responsible for most of the physical and nutritional characteristics of honey. Small quantities of other sugars are also present, such as disaccharides (sucrose, maltose and isomaltose) and a few trisaccharides and oligosaccharides. Though quantitatively of minor importance, their presence can provide information about adulteration and the botanical origin of the honey .(Cooper, 2002)

2:2:3 Water

Water is quantitatively the second most important component of honey. Its content is critical, since it affects the storage of honey. Only honeys with less than 18% water can be stored with little to no risk of fermentation. The final water content depends on a number of environmental factors during production such as weather and humidity inside the hive, but also on nectar conditions and treatment of honey during extraction and storage. It can be reduced before or after extraction by special techniques.(Ferrazzi , 2002)

2:2:4 Organic acids

Among the minor constituents organic acids are the most important and of these gluconic acid, which is a by-product of enzymatic digestion of glucose, predominates. The organic acids are responsible for the acidity of honey and contribute largely to its characteristic taste.

2:2:5 Minerals

Minerals are present in very small quantities, potassium being the most abundant. Dark honeys, particularly honeydew honeys are the richest in minerals.

2:2:6 Nitrogenous compounds

Other trace elements include nitrogenous compounds among which the enzymes originate from salivary secretions of the worker honeybees. They have an important role in the formation of the honey

Their commercial importance is not related to human nutrition, but to their fragility and uniqueness. Thus their reduction or absence in adulterated, overheated or excessively stored honeys serves as an indicator of freshness. The main enzymes in honey are in vertase (saccharase) diastase (amylase) and glucose oxidase.Traces of other proteins, enzymes or amino acids as well as water soluble vitamins are thought to result from pollen contamination in honey virtually absent in newly produced honey, hydroxvmethylfurfural (HMF) is a byproduct of fructose decay, formed during storage or during heating. Thus, its presence is considered the main indicator of honey deterioration.

Even though some of the substances responsible for honey colour and flavour have been identified the majority are still unknown. It is more than likely that honeys from different botanical origins contain different aromatic and other substances which contribute to the specific coloursand flavours and thus allow to distinguish one honey from another. Similarly, it is very likely that, depending on their botanical origin, honeys contain traces of pharmacologically active substances. Some of them have been identified, such as those responsible for the toxicity of certain honeys but for the majority of possible substances, scientific verification requires further studies. .(Stawomir ,2007)

2:2:7 Enzymes

Honey naturally contains small amounts of enzymes that are introduced into honey by the bees during various phases of the honey manufacturing process the predominant enzymes in honey are diastase (amylase), invertase (α-glucosi-dase) and glucose oxidase. Other enzymes such as catalase and acid phosphatase, are generally present in lesser amounts. While enzyme type is fairly uniform across honey varieties the amount of enzyme present can vary Twidely Enzymes play an important role in honey and contribute to its functional properties(Mundo , 2004).

2:3:1 Honey Harvesting and Transport

The harvesting and transport of honey should follow some procedures, aiming at an efficient collection, but mainly to maintain its original characteristics, therefore the quality of the final product.The harvest of honey should not be accomplished in rainy days or when the relative humidity is high, because this would lead to an increased moisture indexes in the honey.

The beekeeper should decided for those hours which there is less air humidity on the sunny days. When harvesting, the beekeeper should not throw smoke directly on the honeycombs; this should be performed at small amounts, by using the bee smoker far away from the frames of honeycombs. These procedures are followed in order to reduce the incorporation of the smokeable smell into both honey and beeswax, as well as detritus from the bee smoker. After harvesting, the frames of honeycombs should not stay exposed to the sun for long periods because high temperatures can lead to the an increased hydroxymetylfurfural content (HMF)in the honey, reduced content of the main enzymes in honey (invertase, glucose oxidase and diastase), therefore endangering the honey quality.

The harvesting and transport of honey should follow some procedures, aiming at an efficient collection, but mainly to maintain its original characteristics, therefore the quality of the final product.

The vehicle used in transporting the supers containing honeycombs to the extracting room should be prepared in the previous day, as subjected to a hygienic process. It is necessary that the vehicle did not recently transport any material that might have left some type of toxic residue, or otherwise has strong odor. Besides, the surface of the vehicle load area should be covered with nontoxic material, properly cleaned and free from impurities, in order to avoid the direct contact of the supers containing honeycombs with the floor. (Goldman, 2014).

2:3:2 Collection

Honey is collected from wild bee colonies, or from domesticated beehives. Wild bee nests are sometimes located by following a honey guide bird. The bees may first be pacified by using smoke from a bee smoker. The smoke triggers a feeding instinct (an attempt to save the resources of the hive from a possible fire), making them less aggressive and the smoke obscures the pheromones the bees use to communicate.

The honeycomb is removed from the hive and the honey may be extracted from that, either by crushing or by using a honey extractor. The honey is then usually filtered to remove beeswax and other debris.

Before the invention of removable frames, bee colonies were often sacrificed in order to conduct the harvest. The harvester would take all the available honey and replace the entire colony the next spring. Since the invention of removable frames, the principles of husbandry lead most beekeepers to ensure that their bees will have enough stores to survive the winter, either by leaving some honey in the beehive or by providing the colony with a honey substitute such as sugar water or crystalline sugar (often in the form of a "candy board"). The amount of food necessary to

survive the winter depends on the variety of bees and on the length and severity of local winters.

2:3:3 Honey filtration

Honey should not be strained with a mesh size smaller than 0.2 mm in order to prevent pollen removal. On the other hand, the shortly revised and EU Directive relating to honey allow a removal of pollen if it is unavoidable for the removal of foreign matter. Such honey should be labelled as "filtered". As microscopical pollen analysis is still the most important tool for the determination of botanical and geographical origin of honey the removal of pollen by filtration will make authenticity testing much more difficult, if not impossible. (Lazaridou,2004).

2:3:4 Preservation

Because of its unique composition and chemical properties, honey is suitable for long-term storage, and is easily assimilated even after long preservation. Honey, and objects immersed in honey, have been preserved for centuries.

The key to preservation is limiting access to humidity In its cured state, honey has a sufficiently high sugar content to inhibit fermentation. If exposed to moist air, its hydrophilic properties will pull moisture into the honey, eventually diluting it to the point that fermentation can begin regardless of preservation, honey may crystallize over time. The crystals can be dissolved by heating the honey. (Aston , 2004)

2:4:1 Adulteration of honey

Adulteration of honey is the addition of other sugars, syrups or compounds into honey to change its flavor, viscosity, make it cheaper to produce, or to increase the fructose content in order to stave off crystallization According to the Codex Alimentarius of the United

Nations, any product labeled as honey or pure honey must be a wholly natural product, although different nations have their own laws concerning labeling.

Adulteration of honey is sometimes used as a method of deception when buyers are led to believe that the honey is pure , The practice was common dating back to ancient times, when crystallized honey was often mixed with flour or other fillers, hiding the adulteration from buyers until the honey was liquefied. In modern times the most common adulteration-ingredient became clear, almost-flavorless corn syrup, which, when mixed with honey, is often very difficult to distinguish from unadulterated honey (CAC, 2001).

2:4:2 Indirect adulteration of honey

Indirect adulteration of honey is accomplished by feeding honeybees with industrial sugars at the stage when broods become naturally available. Such indirect adulteration is extremely difficult to detect

2:4:3 Direct adulteration of honey

Direct adulteration is the addition of foreign substances directly to honey Methods of detecting direct adulteration Traditional analyses of chemical composition and physical properties of honey are commonly used to detect direct adulteration. They are routinely applied in the honey trade but these analytical methods are relatively time-consuming and require tedious preparation of the samples as well as complex analytical equipment (Cozzolino *et al*, 2011). Honey adulteration can also be detected using several modern methods such as measuring stable carbon-isotope ratios, NMR or differential calorimetry. Much attention has been paid to measuring major sugars in honey with gas chromatography (GC) and liquid chromatography coupled to various types of detectors (Abdel-Aal *et al.* 1993; Bogdanov *et al*, 2004).

2:4:4 Adulteration by sweeteners

Adulteration by sweeteners is the most important authenticity issue. As a natural product of a relatively high price, honey has been a target for adulteration for a long time Addition of sweeteners, feeding the bees during the nectar flow or extracting combs obtaining bee feed may adulterate of honey.

The following sweeteners have been detected in adulterated honeys sugar syrups and molasses inverted by acidsor enzymes from corn, sugar cane, sugar beet and syrups of natural origin such as maple Many methods have been tested for adulteration proof but most of them are not capable to detect unequivocally adulteration here only the promising methods are discussed Adulteration by addition of cane- and corn sugar can be screened microscopically Kerkvliet and Meijer (Ferrazzi , 2002).

2:5 Effect of adulteration on honey properties

Honey adulteration is a complex problem, which has a significant economic impact; it can be occurred by the addition of different materials. Adulteration, or the addition of foreign substances to honey such as; molasses, starch solution, glucose, sucrose, water and inverted sugar, were studied by Physiochemical characteristic of Egyptian honey were studied by (Ibrahim *et al*, 1978) adulteration usually refers to mixing other matter (substance) of an inferior and sometimes harmful quality with food or drink intended to be sold. With companies concerned about the bottom line, the temptation to cheat is considerable, and unfortunately, the adulteration of honey is a serious economic and regulatory problem. As usual, the losers are the consumers and the processor or re-processor seeking to provide a wholesome product that meets regulatory standards. From an economic point of view, food product adulteration can destabilize the market by bringing in unfair competition (Ruoff and Bogdanov, 2004).

2:6:1 Honey adulteration in Sudan

This study was carried out to detect some causes of simulated adulterants intentionally added to the commercial Sudanese honeys. An authentic honey sample was intentionally adulterated with 30% cane syrup, commercial glucose syrup, Gum Arabic and Gamardin separately. Gas liquid chromatograms were produced for the sample before and after adulteration. These chromatograms were compared with those of 20 commercial honey samples. Characteristic chromatograms were generated for the authentic sample compare to each of the adulterated sample. The comparison resulted in detection of adulteration in 4 samples with cane syrup, commercial glucose syrup and unknown materials. The result indicated the need for comprehensive investigation of the commercial honeys and developing simple method of detection of adulteration. (Mohammed, 2007)

2:6:2 Honey Adulteration in Ethiopia

Ethiopia is the largest honey- producing country in Africa and one of the top ten countries in the world Manyparts of the Tigray National Regional State in general and theEastern parts of the region in particular are known for their production of high quality of honey in Ethiopia the amount of honey produced in the Tigray Region is almost 15% of the global production of Ethiopian honey. An attempt was made to assess the physicochemical properties of some Tigray honey samples for their electrical conductivity, ash content, moisture content, pH value and elemental composition of some selected metals The physiochemical characteristics of honey obtained from traditional and modern hive production systems in Tigray region have been also compared Nevertheless there is no sufficient work on quality determination as well as effect and extent of adulteration for locally produced natural honey nowadays, adulteration of different natural food items becomes a common problem in many parts of the world regardless of economic status of the nation. One of the susceptible food

materials for intentional or unintentional adulteration or contamination is honey. The most common adulteration practiced with honey is the addition of sucrose, corn syrupmolasses, banana or other harmless or harmful materials. The act of honey adulteration is causing severe impact on the domestic and international market opportunities of the productand may result nutritional and health problems on consumers

.Honey is declared adulterated if cheaper or inferior substances are substituted wholly or in part. It may also contain some added substances injurious to health and for whatever reasons its quality is below the standard. In this work, honeysamples from apiary sites and selected household beekeepersas well as from local markets were collected and assessed for their quality parameters. More over, susceptible adulterants were checked for their effect on the test parameters and simple methods were suggested to screen adulterated honeysamples from the pure ones.(Cooper, 2002).

2:6:3 Honey adulteration in Nigeria

Honey is the natural sweet substance produced by honey bees from the nectar of plants, which bees collect transform by combining with specific substances of their own, deposit, hydrate, store and leave in honeycomb to ripen and mature Freshly collected honey is a viscous liquid that has a greater density than water, relatively low heat conductivity, a low surface tension and various colours that are basically all nuances of yellow amber The history of the use of honey is parallel to the history of man and in virtually every culture evidence can be found of itsuse as a food source and as a symbol employed in religious, magic and therapeutic ceremonies The composition of honey depends highly on the type of flowers utilized by bees, climatic conditions in which the plants grow and mature.

Adulteration involves adding external chemical substance into a food product that contains naturally similar substanceHoney adulteration appeared on the world market in the 1970s when high fructose corn syrup was introduced by the food industry,in nigeria, the consumption

of honey as food has increased considerably in recent years due to changing trends. So far, no data has been reported for honeyproduced in Edo State. (Omode, 2008).

2:6:4 Honey adulteration in Australian

As a natural product of a relatively high price, honey has been a prime target for adulteration reasons of economic gain for a long time Adulteration or the addition of foreign substances to honey is a reprehensible practice, which consists of incorporating sugar syrups into the genuine product .For honey to be considered adulterated, the concentration of the adulterant should be at least 7% Many foreign substances could be added to adulterate honey such as; molasses, starch solution, commercial glucose, sucrose, water, chalkm gelatins, inverted sugar and other substances the use of excessive heat for liquefaction or pasteurization has adverse effects on honey quality. (Rossmann, *et al*, 1992).

2:7:1 **Meat preservation**

Meat preservation in general (of livestock, game, and poultry), is the set of all treatment processes for preserving the nutritious properties, taste, texture, and color of raw, partially cooked, or cooked meats while keeping them edible and safe to consume. Curing has been the dominant method of meat preservation for thousands of years, although modern developments like refrigeration and synthetic preservatives are now beginning to complement and supplant it(Ahmed,2003.)

2:7:2 Effect of bee honey on meat preservation

Meat preservation has allowed safe and plentiful access to the nutrient dense food of meat It has prevented countless food poisoning cases and prevented malnutrition for thousands of years since the 20th century, with respect to the relationship between diet and human disease (e.g. cardiovascular, etc.), scientists have conducted studies on the effects of

lipolysis on vacuum-packed or frozen meat. In particular, by analyzing entrecotes of frozen beef during 270 days at −20 °C (−4 °F), scientists found an important phospholipase that accompanies the loss of some unsaturated fat n-3 and n-6, which are already low in the flesh of ruminants.

In 2015, the International Agency for Research on Cancer of the World Health Organization classified processed meat, i.e. meat that has undergone salting, curing, fermenting, and smoking, as "carcinogenic to humans.(Chawla ,2006).

2:7:3 Sugar

The sugar added to meat for the purpose of curing it comes in many forms, including honey, corn syrup solids, and maple syrup. However, with the exception of bacon, it does not contribute much to the flavor ,but it does alleviate the harsh flavor of the salt. Sugar also contributes to the growth of beneficial bacteria such as Lactobacillus by feeding them.

2:7:4 Curing

Is any of various food preservation and flavoring processes of foods such as meat, fish and vegetables, by the addition of combinations of salt, nitrates, nitrites, and/or sugar, with the aim of drawing moisture out of the food by the process of osmosis Many curing processes also involve smoking, spicing, or cooking. Dehydration was the earliest form of food curing.

Because curing increases the solute concentration in the food and hence decreases its water potential, the food becomes inhospitable for the microbe growth that causes food spoilage. Curing can be traced back to antiquity, and was the primary way of preserving meat and fish until the late 19th century. (Chambers , 2001).

CHAPTER THREE

Materials and Methods

3.1 Collection of samples:

Ripe bee honey samples were collected from four states: South Darfur, South Kordofan, Omdurman market and River Nile. The study was conducted under laboratory conditions at College of Agricultural studies (CAS), Sudan University of Science and Technology (SUST) and Central Laboratory in the University of Khartoum during February to April 2016 to study the adulteration of Sudanese bee honey.

3.2. Determination of Glucose and Fructose content (%):

One ml of honey sample from defferent States was mixed with 9mls of distilled water. This was shaken vigorously to dissolve and the mixture was then centrifuged. The clear solution (20µl) was injected with a microsyringe into High Performance Liquid Chromatography (HPLC) analysis. The run time was 20minutes. (Adebiyi, 2004) The HPLC used was Agilent 1200 series.

3.3 Determination of moisture content:

The moisture content of each honey sample was determined by measuring 5g of the sample and placed into a pre- weighted aluminum drying dish. The sample was dried to constant weight in an oven at 105°C for 6 h.

Moisture content (%) =

$$\frac{M2 - M3}{M2 - M1} \times 100$$

Where:

M_1 = mass of dish + cover.

M_2 = mass of dish + cover + sample before drying.

M_3 = mass of dish + cover + sample after drying.

3.4 Determination of Total Titratable A cidity:

Diluted five gram of each sample was titrated against 0.1 N NaOH using mL phenolphthalein as an indicator. The relative amount of citric acid was determined using the mathematical formulae:

$$\text{Titratable acidity} = \frac{(\text{N NaoH}) \times (\text{mls NaOH}) \times \ 0.64}{\text{Weight of sample}} \times \ 100$$

Were:

N = Normality of NaOH.

0.9 = Factor of citric acid.

3.5 . Determination of ash content:

Three gram of each honey sample was separately weighted out into a porcelain crucible previously ignited and weighted. Organic matter was charred by igniting the sample on a hot plate in the fume cupboard. The crucible were then placed in the in the muffle furnace and maintained at 600°C for 6 h. They were then cooled in a desiccator and Weighted immediately (AOAC, 1990) the percent Ash was calculated as:

$$\text{Ash content (\%)} = \frac{\text{W1} - \text{W2}}{\text{Weight of sample}} \times 100$$

Where:

W1 = Weight of crucible with ash.

W2 = Weight of empty crucible.

3:6. Desgin And Astatisical Analysis :

All expermints were arraned in at complete rondomize disgn. Data were subjected to analysis of variance (ANOVA).Least signifint differences (LSD) test was usad for mean seperation.

CHAPTER FOUR

RESULTS

4.1 The First Experiment :

Determination of Moisture Content of Sudanese Bee Honey

The moisture content of each honey sample was determined by slice meat preservation samples which were putting in plastic dishes and weighted during five days(table 3) were result obtain .

Table. 1: Determination of Moisture Content of different Sudanese Bee Honey.

Treatment Samples	Samples Weighted(mg)				
	Exposure time (hrs)				
	W1(24h)	W2(48h)	W3(72h)	W4(96h)	W5(120h)
C	7.62^{A}	7.5^{A}	8.04^{A}	6.4^{A}	5.7^{A}
S.K	7.52^{A}	6.4^{A}	6.5^{AB}	6.2^{A}	4.6^{A}
R.N.S	6.72^{A}	7.22^{A}	6.6^{AB}	5.7^{A}	5.6^{A}
S.D	6.04^{A}	6.5^{A}	7.16^{AB}	5.5^{A}	5.02^{A}
D.W	6.8^{A}	6.6^{A}	7.7^{A}	5.6^{A}	5.06^{A}
OM.M	6.2^{A}	6.7^{A}	5.8^{B}	5.7^{A}	5.16^{A}
SE	0.8786	0.8518	0.8203	0.8124	0.756
CV%	20.38	19.7	18.5	22.11	22.91

Means followed the same letter are not significantly different at($p<0.05$).

Keys: C: Control OF Treatment / S.K: South Kordofan / R.N.S: River Nile State

S.D: South Darfur / D.W : Distilled Water / OM.M: Omdurman Market

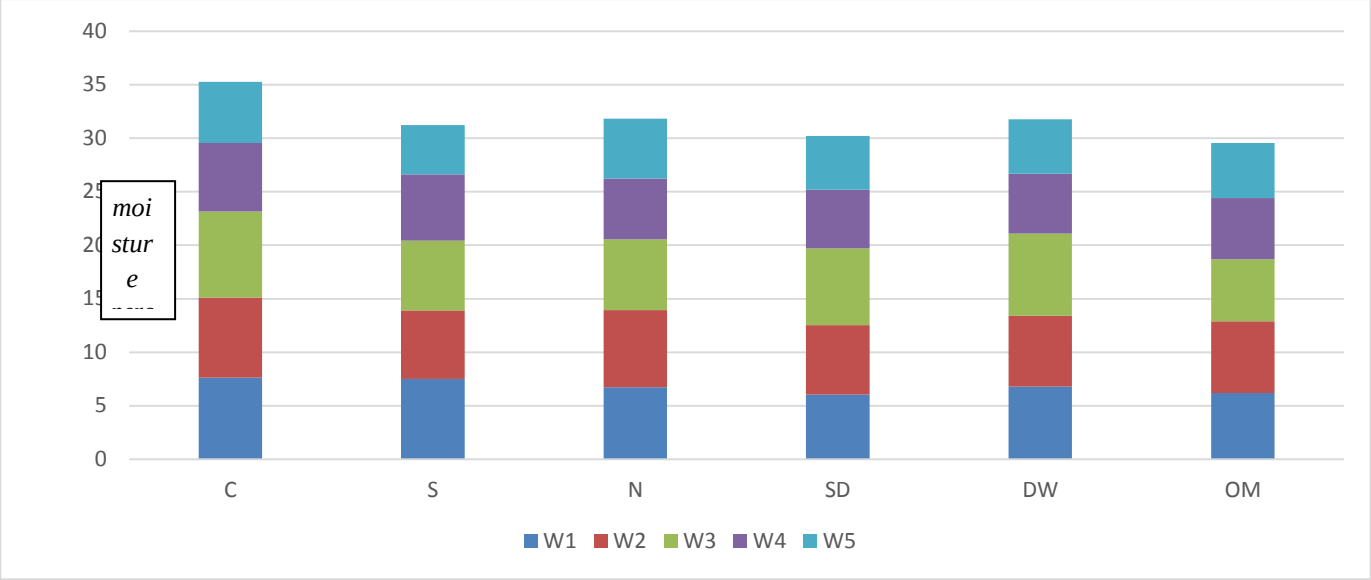

Fig.1: The moisture percentage to different honey samples

4:2 Second Experiment:

Chemical Analysis of the Sugar Content in Sudanese Bee Honey.

Table. 2: Sugar Content on different Sudanese Bee Honey

Areas / States	Maltose	Sucrose	Glucose	Fructose
	g/50 ml	g/50 ml	g/50 ml	g/50 ml
S.K	0	0	6.99^{A}	13.04^{A}
S.D	0	0	5.81^{A}	9.26^{A}
R.N.S	0	0	6.98^{A}	13.85^{A}
OM.M	0	0	15.15^{A}	26.45^{A}
SE	0	0	2.15	3.7
CV%	0	0	2.9	4.2

Means followed the same letter are not significantly different at($p<0.05$).

Key:

S.K: South Kordofan R.N.S: river nail state S.D: South Darfur OM.M: Omdurman Market

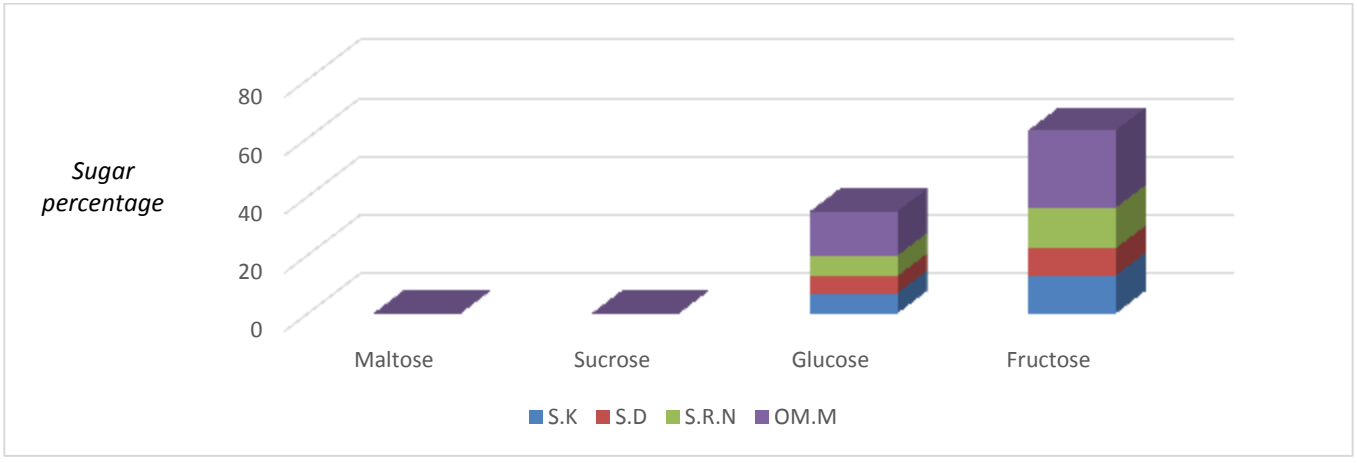

Fig.2: The Sugar content percentage to different honey samples

Table . 3: Chemical anlysis of sudanese bee honey samples

Areas/States	PH	Acidity%	Moisture%	Ash%
S.K	0.78^{A}	0.075^{A}	6.6665^{A}	0.0325^{A}
S.D	0.8175^{A}	0.05^{A}	8.33325^{A}	0.0025^{A}
R.N.S	0.835^{A}	0.125^{AB}	8.33325^{A}	0.965^{B}
OM.M	0.745^{A}	0.025^{AB}	3.6665^{B}	0.215^{AB}
SE	0.020	0.021	1.10	0.22
CV%	0.0016	0.0018	4.84	0.203

Means followed the same letter are not significantly different at($p<0.05$).

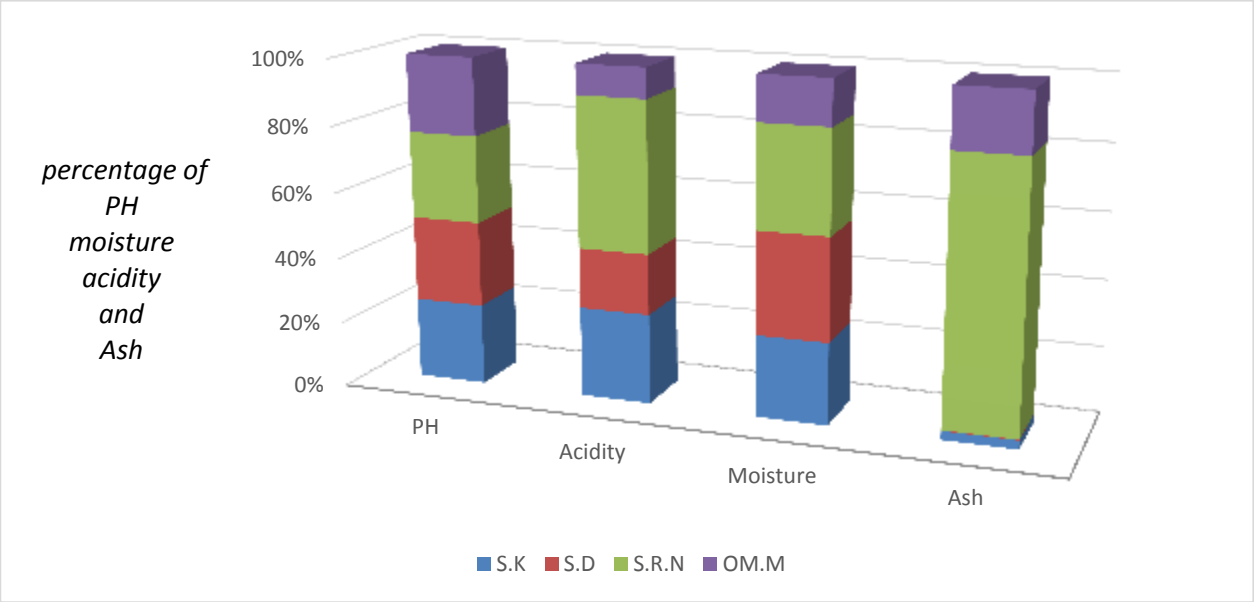

Fig. 3: The percentage of PH , Acidity ,Moisture and ASH

CHPTER FIVE

DISCUSSION

The reason for testing honey for quality control purposes is to reveal the possible presence of artificial components or adulterants, as well as to address processing and market needs. This requires not onlydetermining . (Bogdanov, Stefan, 2009).

The moisture content in first experment not apparent variations in replicate besides some samples(Omdurman market 6.7%),(South.Kordofan. 6.4%) and weithet excess in (Control . 7%.5%),(River Nile State. 7.22%).vide table No (3)and figer No(1)

Thepercentage of sugars contentfirstly not detect sugar maltose and sucrose by (HPLC) whereas , and during from expriment on detect sugar percentage were results differently inasmuch between bee honey samples , the glucose high percentage in (Omdurman market15.15 %) and very low in (River Nile State6.98 %,South Darfur 5.81%), intermediately in (South.Kordofan 6.99%) and variations were fructose percentage in some samples high percentage in (Omdurman market 26.45% , River Nile State 13.85%) intermediately in(South Darfur, 9.26% South.Kordofan13.4%)

The percentage of PH the lower in (South.Kordofan .078%) ,(Omdurman market .0.745%)and the same in (South Darfur .0.8175%),(River NileState .0.745).

Thepercentage of acidity there are apparent variations samplesbetween percentage high in (River Nile State 0.125%). and less in (South Darfur 0.05%), (Omdurman market)

Thepercentage of moisture is lower in(Omdurman market 3.667%), (South.Kordofan 6.667%), and convergentapparently in (South

Darfur8.334%),(River Nile8,334%); (South Darfur),(South.Kordofan),very high in (River NileState).determining the moisture resulting is comparative with stander in table No (1) the seeming convergence in percentage samples See table No (5)honeys samples in this study as all the honey samples had very different moisture content ranging from 8.33325% to 3.6665%.

Thepercentage of ash content during from statistical analysis purity percentage high in (South Darfur 0%); (South.Kordofan 0%) middle in (Omdurman market 5%) very low in (River NileState 9%).

CONCLUSION AND RECOMMMENDATIONS

This study was conducted for the purpose of detecting adulteration on some Sudanese bees honeysamples which included four different directions were clear differences between them in the moisture percentage , purity honey samples and proportions of sugars content, from during the treatment existed in best Omdurman market sample and near the world standard therefore we refer to be as the following :

1. Honey producers exhorted the importance of honesty in supply and demand

2.The State stimulate beekeeping to produce honey and facilitate the marketed locally and globally

3.The specifications and standards setting known and recognized standard for determining the quality of Sudanese bees honey

4.Recommendation for the Ministry of Agriculture - take care of the bee colonies and its economic importance of national income.

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APPENDICES

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شهادة تحليل

Appendix . 1 : Detected the sugars ratio in bees honey sample

Areas / States	**Maltose g/50 ml**	**Sucrose g/50 ml**	**Glucose g/50 ml**	**Fructose g/50 ml**
S.K	ND	ND	27.945	52.143
S.D	ND	ND	23.235	37.04
S.R.N	ND	ND	27.915	55.4
OM.M	ND	ND	60.5915	105.79

Key: ND: Not Detected

Sample Information Acquired by

Sample Name : Admin

Sample ID

Tray# : 1

Vail# : 4

Injection Volume : 20 uL

Data Filename : ho fawaz sue std.lcd

Method Filename : honey agri.lcm

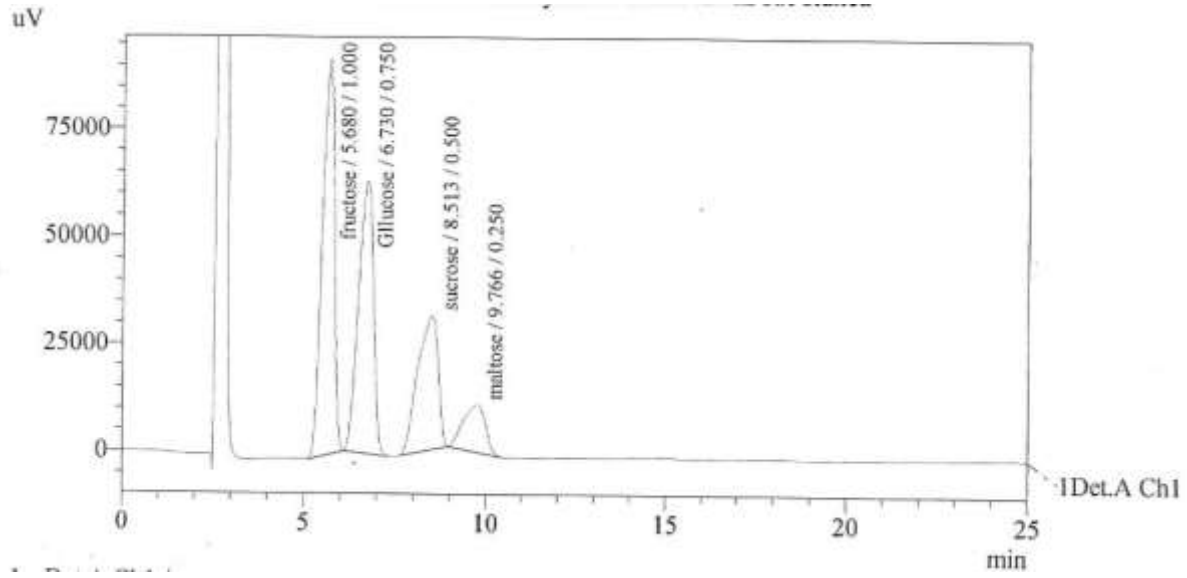
Batch Filename : banana & salma batch.lcb

Report Filename : Default.ler

Date Acquired : 30/09/36 06:38:56 ص

Data Processed : 30/09/36 08:23:38 ص

Chromatogram
E:\Amino Acid Analysis for Nallo fawaz suc std.lcd



1 Det.A Ch l / Peak Table

Detector A Ch1

Appendix.2: Chemical analysis of bee honey

Peak#	Name	Ret. Time	Area	Height
1	Fructose	5.680	2118401	92107
2	Glucose	6.730	1844276	63426
3	Sucrose	8.513	1218298	31380
4	Maltose	9.766	470161	11129
Total			551136	198042

Conc.
1.00
0.750
0.500
0.250

Sample Information Acquired by : Admin

Sample Name

Sample ID

Tray# : 1

Vail# : 15

Injection Volume : 20 uL

Data Filename : honey reim std.lcd

Method Filename : honey agri.lcm

Batch Filename : honey reim.lcb

Report Filename : Default.lcr

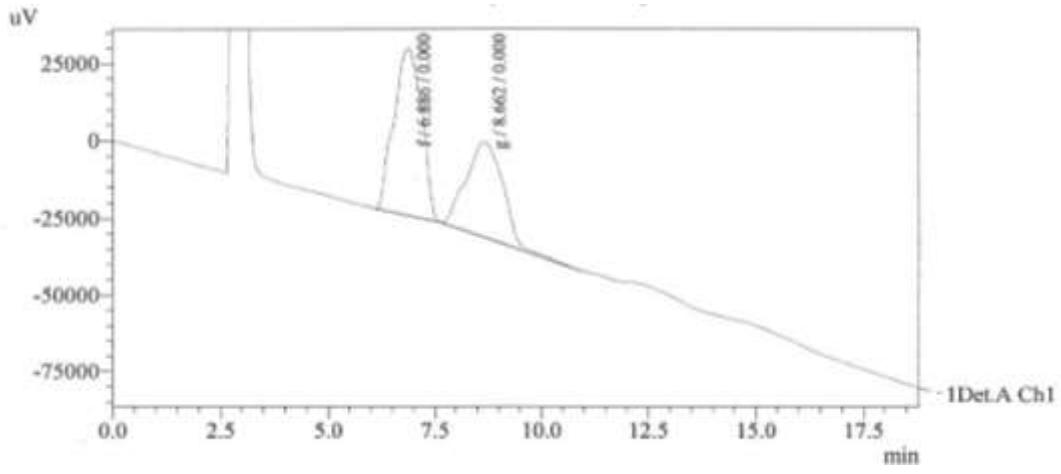
Date Acquired : 22/03/26 01:49:33 ص

Data Processed : 22/03/26 02:37:28 ص

Chromatogram

E:\Amino Acid Analysis for Nathoney reim std.lcd

LIV



1 Det. A Ch/

Peak Table

Detector A Ch1

Appendix.3: Chemical analysis of bee honey

Peak#	Name	Ret. Time	Area	Height
1	F	6.886	2445896	54235
2	G	8.662	1861341	30763
Total			4307237	84999

Conc.
0.000
0.000

Sample Information Acquired by

Sample Name : Admin

Sample ID

Tray# : 1

Vail# : 11

Injection Volume : 20 uL

Data Filename : honey reim. .1..1cd

Method Filename : honey agri.lcm

Batch Filename : reim.lcb

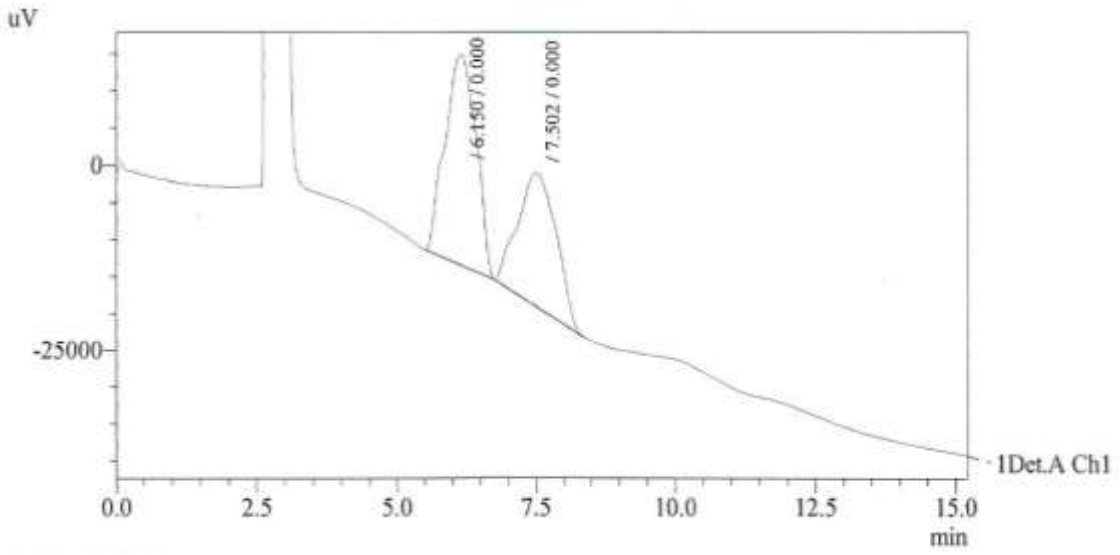
Report Filename : Default.lcr

Date Acquired : 22/03/26 02:26:03 u

Data Processed : 22/03/26 01:56:57

Chromatogram

E:\Amino Acid Analysis for NaThoney reim. .1..lcd



1Det.A Chl

PeakTable

Detector A Chi

Appendix.4: Chemical analysis of bee honey

Peak#	Name	Ret. Time	Area	Height
1	Fructose	6.150	1104595	28340
2	Glucous	7.502	867911	18016
Total			1972506	46356

Conc.
0.52143
0.27975

Sample Information Acquired by

Sample Name: Admin

Sample ID

Tray# : 1

Vail# : 12

Injection Volume : 20 uL

Data Filename : honey reim 2.1cd

Method Filename : honey agri.lcm

Batch Filename : reim.lcb

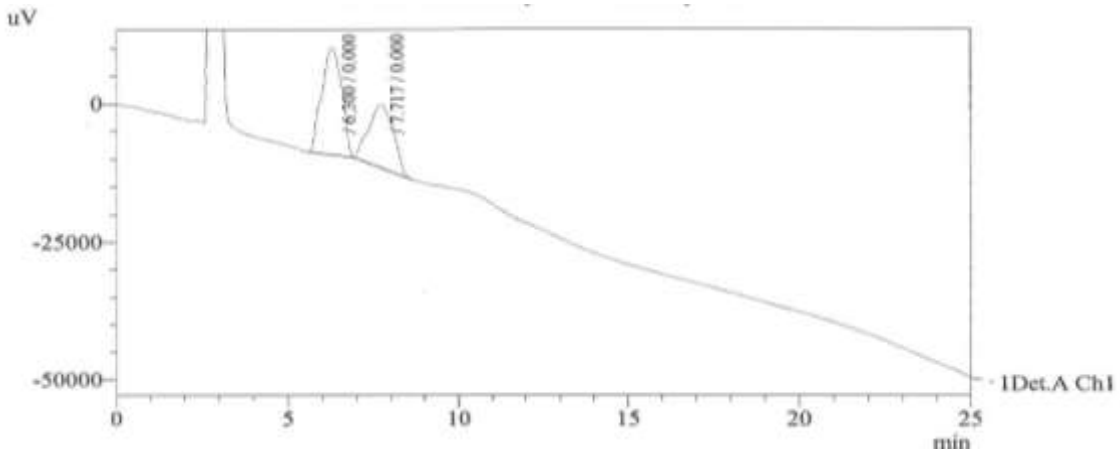
Report Filename : Default.lcr

Date Acquired : 22/03/26 01:41:40

Data Processed : 22/03/26 01:56:58

Chromatogram

E:\Amino Acid Analysis for NaThoney reim 2.lcd



1 Det. A Ch1/

PeakTable

Detector A Chi

Appendix.5: Chemical analysis of bee honey

Peak#	Name	Ret. Time	Area	Height
1	Fructose	6.300	784653	19355
2	Glucous	7.717	571326	11337
Total			1355978	30692

Conc.
0.3704
0.23235

Sample Information Acquired by : Admin

Sample Name

Sample ID

Tray# : 1

Vail# : 14

Injection Volume : 20 uL

Data Filename : honey reim4.1cd

Method Filename : honey agri.lcm

Batch Filename : reim.lcb

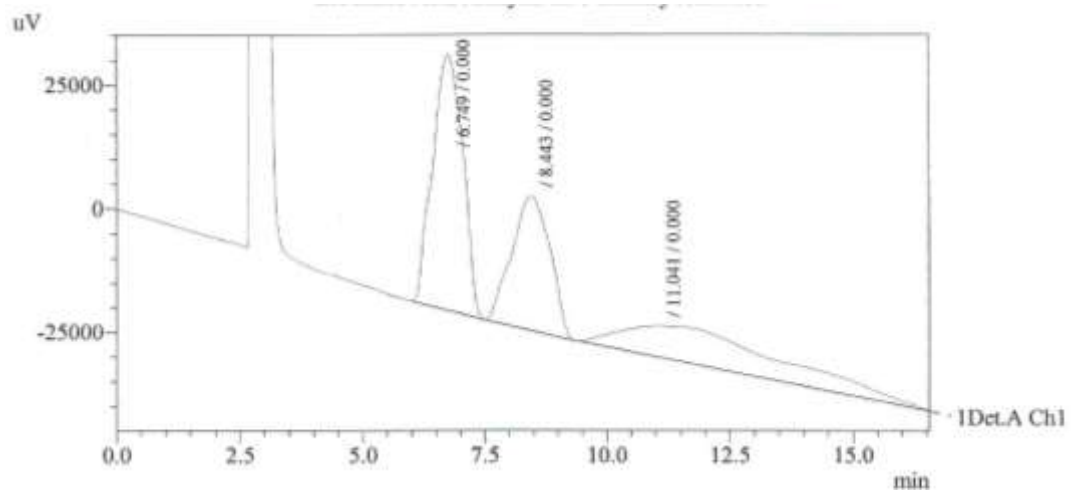
Report Filename : Default.lcr

Date Acquired : 22/03/26 01:32:35ص

Data Processed : 22/03/26 01:56:58 ص

Chromatogram

E:\Amino Acid Analysis for Na\honey reim4.1cd



1 Det. A Ch1/

Peak Table

Detector A Chi

Appendix.6: Chemical analysis of bee honey

Peak#	Name	Ret. Time	Area	Height
1	Fructose	6.749	2241038	51721
2	Glucous	8.443	1490148	27196
3		11.041	1631363	6242
Total			5362549	85158

Conc.
0.3704
0.23235

Sample Information Acquired by : Admin

Sample Name

Sample ID

Tray# : 1

Vail# : 13

Injection Volume : 20 uL

Data Filename : honey reim 3.Icd

Method Filename : honey agri.lcm

Batch Filename : reim.lcb

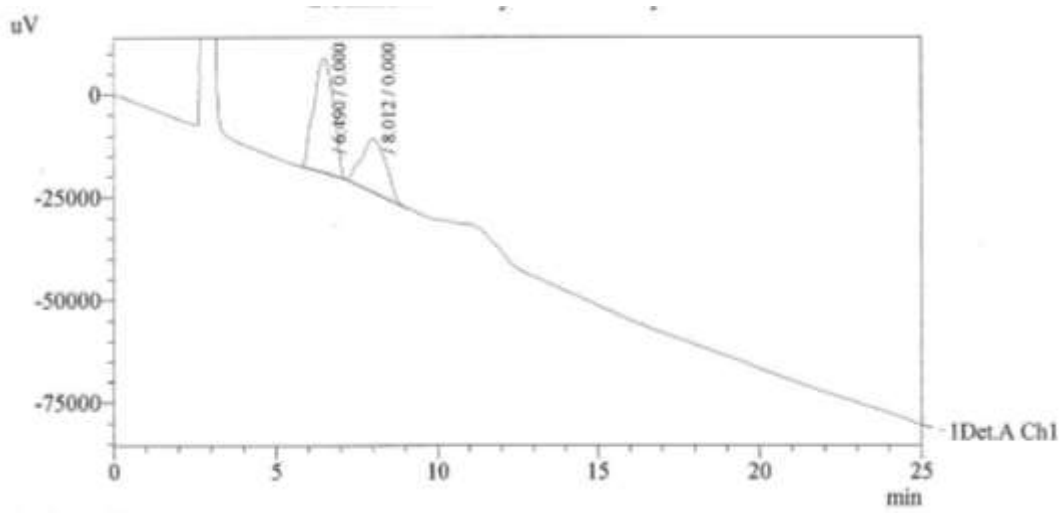
Report Filename : Default.lcr

Date Acquired : 22/03/26 02:07:08 ص

Data Processed : 22/03/26 01:56:58ص

Chromatogram

E:\Amino Acid Analysis for NaThoney reim 3.Icd



1 Det. A Ch1/

Peak Table

Detector A Chi

Appendix.7: Chemical analysis of bee honey

Peak#	Name	Ret. Time	Area	Height
1	Fructose	6.490	1173618	27912
2	Glucous	8.012	686367	12912
Total			1859985	40823

Conc.
0.5540
0.27915

Appendix:8. Explicates the percentages in sugars content in Sudanese Bee Honey

Areas / States	Maltose	Sucrose	Glucose	Fructose	Total
	g/50 ml	g/50 ml	g/50 ml	g/50 ml	
S.K	0	0	35%	65%	100%
S.D	0	0	39%	61%	100%
R.N.S	0	0	34%	66%	100%
OM.M	0	0	36%	64%	100%

Appendix :9. Explicates the percentages in PH , Acidity , Moisture , and

Areas/States	PH	Acidity	Moisture	Ash	total
S.K	10%	1%	88%	0%	100%
S.D	9%	1%	91%	0%	100%
S.R.N	8%	1%	81%	9%	100%
OM.M	16%	1%	79%	5%	100%

Ash

Plate 1-Bee Honey Samples

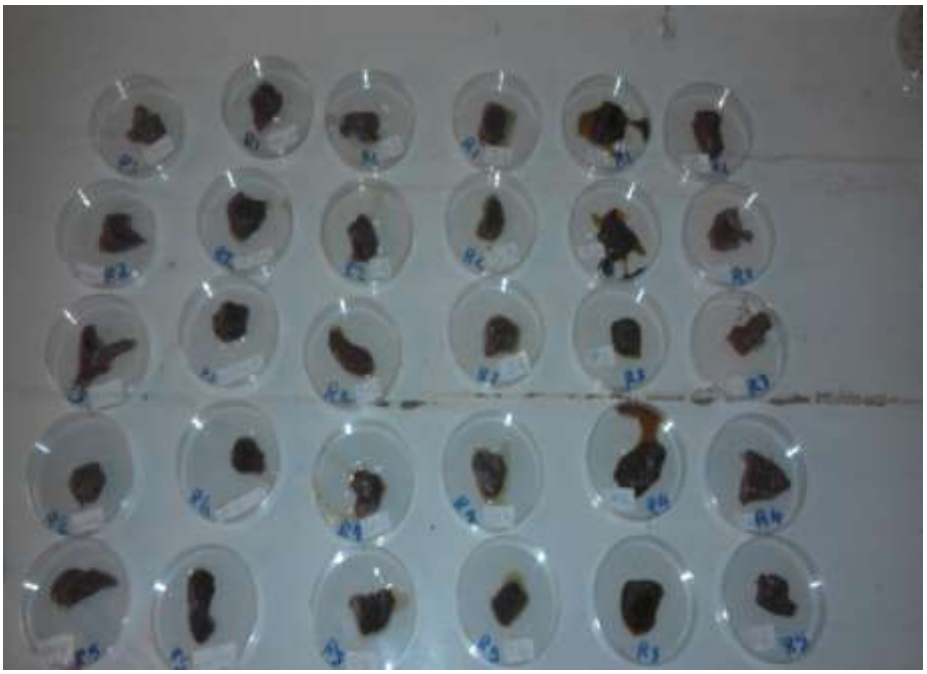


Plate 2- Pieces of meat

Plate 8-Crucible contained ash of samples

Plate 9- Aluminum Dishes to determine moisture content