Some Physicochemical Characteristics of Natural Bee Honey 
And Tow Commercial Types of Honey

By
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B.Sc. (Hon) in Food Engineering and Technology
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April ,2013
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Date: April /2014
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Examination Committee

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يقول الله تبارك وتعال:

وَأَوْحَى رَبُّكَ إلَى النَّحْلِ أَنِ اتَّخِذِي مِنَ الجِبَالِ بُيُوتًا وَمِنَ الشَّجَرِ وَمِمَّا يَعْرِشُونَ * ثُمَّ كُلِي مِن الثَّمَرَاتِ فَاسْلُكِي سُبُلَ رَبِّكِ ذُلُلاً يَخْرُجُ مِن بُطُونِهَا شَرَابٌ مُّخْتَلِفٌ أَلْوَانُهُ فِيهِ شِفَاءٌ لِّلنَّاسِ إنَّ فِي ذَلِكَ لآيَةً لِّقَوْمٍ يَتَفَكَّرُونَ

(سورة النحل آية 68-69)
Dedication

For

Aboode

And

Lojain

And

Aaya

And

All my Family
ACKNOWLEDGEMENT

Above all, I render my thanks to the merciful "Allah" who offered me health and patience to accomplish this study.

special thank to my Husband Haitham Abd EL moinem Mohamed

I also extend my sincere thanks to my mother, my father and my big family for support and constant encouragement.

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Finally, Iam also grateful to all the staff of the college library of University of Gezira, and all help me of this study
Some Physicochemical Characteristics of Natural Bee Honey and Two Commercial Types of Honey

Amel Ibrahim Abd Alrahman Abd allah

Abstract

Honey is a natural food and a trusted medicine, composed of a complex mixture of carbohydrates and other minor substances, such as organic acids, amino acids, proteins, minerals, and vitamins. The objective of this work was to study some physicochemical characteristics of natural bee-honey and two other commercial samples of honey. Three samples of honey were obtained from Wad Medani local market. Samples included natural honey of bees (HB). Industrial black honey (BH) (El Bawadi, made in Egypt) and white honey (WH) (Lyle’s golden Syrup, made in United kingdom). Some physicochemical characteristics (density, refractive index, viscosity and pH), approximate analysis (moisture, ash, protein and carbohydrates content). Minerals, energy and carbohydrates (total soluble sugar and total solids) were determined for each of the honey samples. The obtained data were subjected to an appropriate data analysis. The results of this work revealed that, the standard pH value for honey was relatively closer to that of HB and less than those of WH and BH while that the obtained values of refractive index for the three types of honey were within the range of honey standards. The moisture content of the three samples was more than the USA standards, while the different samples of honey had relatively similar ash content. Although contents of protein were too low, but it still within the standards of quality. Total soluble sugar (TSS) content of the three types of honey also was within the recommended range. The mineral contents in HB observed to be more than that of WH and BH. The richest product with energy was the BH followed by the HB and lastly the WH. The highest percentage of soluble sugars (SS) was found in the WH, followed by HB, while the lowest value was in the BH. The results showed that the percentage total soluble solid (TSS) was higher in HB sample, followed by WH and lastly BH sample. This study recommends to run complete tests on the imported commerce honey in reference to the standards and to determine the factors affecting its properties.
العسل هو غذاء طبيعي وعلاج موثوق يتكون من معقد من الكربوهيدرات ومواد أخرى بكميات قليلة، مثل الإحماض العضوية والأمينية، البروتينات، العناصر المعدنية، والفيتامينات. هدف هذا البحث دراسة بعض الخصائص عسل النحل الطبيعي ونوعين من العسل التجاري. احضرت عينات العمل الثلاثة من السوق المحلي لمدينة ود مدني، وشملت العينات عسل النحل الطبيعي، العسل الأسود الصناعي (عسل البوادي، صنع في جمهورية مصر العربية) والعسل الأبيض عسل لي ليز الذهبي، صنع في المملكة المتحدة. تم تحديد بعض الخصائص الفيزيوكيميائية (الكثافة، معامل الإنكسار، اللزوجة والأس الهيدروجيني)، التحليل التقريبي (نسبة الرطوبة، الرماد، البروتين، والكربوهيدرات)، العناصر المعدنية، الطاقة والكربوهيدرات (السكريات الذائبة الكلية والمواد الصلبة الكلية). في كل عينة أوضحت نتائج هذا البحث من عينات العسل الثلاثة. أخذت بيانات التجربة لتحليل إحصائي مناسب. أوضحت نتائج هذا البحث أن قيمة درجة الأس الهيدروجيني القياسية للعسل قريبة نسبياً من قيمة الأس الهيدروجيني للعسل الطبيعي وأقل من قيمة العينتين الأخريين للعسل، في حين أن قيمة معامل الإنكسار لعينات العسل الثلاثة تقع ضمن مدى القياس. وجد أن الرطوبة للعينات الثلاثة أكثر من المعايير الأمريكية، في حين أن محتوى الرماد، متساوي في العينات الثلاثة، وعلى الرغم من أن محتوى البروتين كان قليل جداً، لكنه يبقى ضمن مدى معايير الجودة. السكريات الذائبة الكلية في الثلاثة عينات تقع أيضاً في مدى المصايف، ولاحظ أن محتوى العناصر المعدنية في العسل الطبيعي أكثر مقارنة بالعسل الأسود والأبيض. المنتج الأغلى بالطاقة هو العسل الأسود ثم العسل الطبيعي وأخيراً العسل الأبيض. وجاءت أعلى نسبة مئوية للسكريات الذائبة في العسل الأبيض ثم العسل الطبيعي بينما العسل الأسود كان أقل قيمة. أوضحت النتائج أن نسبة المواد الصلبة الذائبة كانت أكثر في عينة العسل الطبيعي ثم العسل الأبيض وأخيراً في عينة العسل الأسود. توصي هذه الدراسة بإجراء اختبارات كاملة لعينات العسل التجاري المستورد ومقارنتهم مع العسل الطبيعي، وكذلك العسل الذي تؤثر على خصائصه.
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CHAPTER ONE
INTRODUCTION

Honey is a natural food, mainly composed of a complex mixture of carbohydrates and other minor substances, such as organic acids, amino acids, proteins, minerals, and vitamins. In almost all honey types, fructose predominates, glucose being the second main sugar. These two account for nearly 85–95% of the honey carbohydrates. More complex sugars made up of two or more molecules of glucose and fructose constitute the remaining carbohydrates, except for a trace of polysaccharide. Honey also contains volatile substances which are responsible for the characteristic flavor. The Codex Alimentarius Standard for honey quality includes several chemical and physical parameters, comprising moisture content, mineral content, acidity, hydroxyl methyl furfural content, diastase activity, apparent sugar content, and water insoluble solids content. These analyses help the food analyst to determine the “chemical” quality of the honeys analyzed. Moreover, suggest that they may be used in association with multivariate analyses to assign floral origin. The honey analyses conductive pH free acidity and percentages of fructose, glucose and raffinose as variables for the principal component analysis (Finola, 2007).

Honey is very hygroscopic, which means that it easily absorbs moisture from the air. Thus, in areas with a very high humidity it can be difficult to produce good quality honey of sufficiently low water content. Honey constitutes a complex food matrix containing small amounts of a high number of floral nectar phytochemicals that are transformed by the bee saliva enzymes and during the honey maturation process in the hive. In addition, it can contain other plant-derived metabolites as is the case of pollen and propolis (plant exudates and resins) constituents. These plant compounds can be used for geographical and floral origin determinations of honey.

Honey is color graded into light, amber, and dark categories which do not really have any bearing on quality. Some of the most distinctively and strongly flavored honey varieties, such as basswood, are very light, while very mild and pleasant honeys such as tulip poplar can be quite dark. While it is not an indicator of honey quality and there are exceptions to the rule, generally speaking, the darker color the honey, the higher its
mineral contents, the pH readings, and the aroma/flavor levels. Minerals such as potassium, chlorine, sulfur, iron, manganese, magnesium, and sodium have been found to be much higher in darker honeys (Truchado et al., 2011).

Honey is a natural food of great interest for its high nutritional value and therapeutic and medicinal properties. Api-therapy (the medicinal use of honeybee products) has recently gained attention for preventive medicine in several conditions and diseases as well as for promoting health and well-being. Because of its sweetness, color and flavor, honey is also often used in place of sugar and as an ingredient or natural preservative in many manufactured foods. It can prevent oxidative reactions in foods (e.g. lipid oxidation in meat and enzymatic browning of fruits and vegetables. The composition and properties of a particular honey sample depend strongly on the type of flowers the bees visited, on the climatic conditions in which the plants grow and on the beekeeper's contribution. From the chemical point of view, honey is a highly complex, concentrated mixture of sugars with a large pool of minor constituents of different molecular weights (MW) and chemical nature embedded. Many of these components are thought to be responsible for its beneficial properties, from high-MW components (proteins) excreted by honey bees into honey (e.g. glucose oxidase, invertase, saccharase, diastase and catalase) and, to peculiar and particularly abundant plant secondary metabolites e.g. kynurenic acid (KA) in chestnut honey, cyclohexa-1,3-diene-1-carboxylic acid (CDCA) in lime tree honey, and/or an array of minor compounds (phenolic acid derivatives such as ferulic acid, caffeic acid and coumaric acid and its esters, and flavonoid aglycones) deriving from the original composition of the nectars and sugar-rich materials on which honey bees feed to produce honey (Giangiacomo et al., 2011).

**Objective**

The aim of this work was to study some physicochemical characteristics of bee-honey in comparison to two commercial samples of honey.
CHAPTER TWO
LITERATURE REVIEW

2.1. Honey

Honey is a sweet food made by bees using nectar from flowers. The variety produced by honey bees (the genus *Apis*) is the one most commonly referred to and is the type of honey collected by beekeepers and consumed by humans. Honey produced by other bees and insects has distinctly different properties. Honey bees transform nectar into honey by a process of regurgitation, and store it as a primary food source in wax honeycombs inside the beehive. Beekeeping practices encourage overproduction of honey so the excess can be taken from the colony (Lansing *et al*., 1999).

Honey gets its sweetness from the monosaccharide’s fructose and glucose, and has approximately the same relative sweetness as that of granulated sugar. It has attractive chemical properties for baking, and a distinctive flavor that leads some people to prefer it over sugar and other sweeteners. Most microorganisms do not grow in honey because of its low water activity of 0.6. However, honey sometimes contains dormant endospores of the bacterium *Clostridium botulinum*, which can be dangerous to infants, as the endospores can transform into toxin-producing bacteria in the infant's immature intestinal tract, leading to illness and even death (Shapiro *et al*., 1998).

Honey has a long history of human consumption, and is used in various foods and beverages as a sweetener and flavoring. It also has a role in religion and symbolism. Flavors of honey vary based on the nectar source, and various types and grades of honey are available. It is also used in various medicinal traditions to treat ailments. The study of pollens and spores in raw honey can determine floral sources of honey (Vaughn and Bryant, 2001). Because bees carry an electrostatic charge, and can attract other particles, the same techniques can be used in area environmental studies of radioactive particles, dust or particulate pollution (Mercuri and Porrini, 1991).

2.1.1. Formation

Honey is produced by bees as a food source. In cold weather or when fresh food sources are scarce, bees use their stored honey as their source of energy. By contriving
for bee swarms to nest in artificial hives, people have been able to semi-domesticate the insects, and harvest excess honey. In the hive (or in a wild nest), there are three types of bee in a hive: a single female queen bee, a seasonally variable number of male drone bees to fertilize new queens, and some 20,000 to 40,000 female worker bees. The worker bees raise larvae and collect the nectar that will become honey in the hive. Leaving the hive, they collect sugar-rich flower nectar and return (Val, 2007).

In the hive, the bees use their "honey stomachs" to ingest and regurgitate the nectar a number of times until it is partially digested. The bees work together as a group with the regurgitation and digestion until the product reaches a desired quality. It is then stored in honeycomb cells. After the final regurgitation, the honeycomb is left unsealed. However, the nectar is still high in both water content and natural yeasts, which, unchecked, would cause the sugars in the nectar to ferment. The process continues as bees inside the hive fan their wings, creating a strong draft across the honeycomb, which enhances evaporation of much of the water from the nectar. This reduction in water content raises the sugar concentration and prevents fermentation. Ripe honey, as removed from the hive by a beekeeper, has a long shelf life, and will not ferment if properly sealed (Standifer, 2007).

2.1.2. Physical Properties

The physical properties of honey vary, depending on water content, the type of flora used to produce it, temperature, and the proportion of the specific sugars it contains. Fresh honey is a supersaturated liquid, containing more sugar than the water can typically dissolve at ambient temperatures. At room temperature, honey is a super-cooled liquid, in which the glucose will precipitate into solid granules. This forms a semisolid solution of precipitated sugars in a solution of sugars and other ingredients (Root, 2005).

The melting point of crystallized honey is between 40 and 50°C (104 and 122 °F), depending on its composition. Below this temperature, honey can be either in a metastable state, meaning that it will not crystallize until a seed crystal is added, more often, it is in a "labile" state, being saturated with enough sugars to crystallize spontaneously. The rate of crystallization is affected by the ratio of the main sugars, fructose to glucose, as well as the dextrin content. Temperature also affects the rate of crystallization, which
is fastest between 13 and 17 °C (55 and 63 °F). Below 5 °C, the honey will not crystallize and, thus, the original texture and flavor can be preserved indefinitely (Piotr, 2004).

Since honey normally exists below its melting point, it is a super cooled liquid. At very low temperatures, honey will not freeze solid. Instead, as the temperatures become colder, the viscosity of honey increases. Like most viscous liquids, the honey will become thick and sluggish with decreasing temperature. While appearing or even feeling solid, it will continue to flow at very slow rates. Honey has a glass transition between -42 and -51 °C (-44 and -60 °F). Below this temperature, honey enters a glassy state and will become a non crystalline amorphous solid (Kántor et al., 1999).

The viscosity of honey is affected greatly by both temperature and water content. The higher the humidity, the easier honey will flow. Above its melting point, however, water has little effect on viscosity. A side from water content, the composition of honey also has little effect on viscosity, with the exception of a few types. At 25 °C (77 °F), honey with 14% humidity will generally have a viscosity of around 400 poise, while a honey containing 20% humidity will have a viscosity of around 20 poise. Viscosity increase due to temperature occurs very slowly at first. A honey containing 16% humidity, at 70 °C (158 °F), will have a viscosity of around 2 poise, while at 30 °C (86 °F), the viscosity will be around 70 poise. As cooling progresses, honey will become more viscous at an increasingly rapid rate, reaching 600 poise around 14 °C (57 °F). However, while honey is very viscous, it has rather low surface tension (Root, 2005).

A few types of honey have unusual viscous properties. Honey from heather or manuka display thixotropic properties. These types of honey enter a gel-like state when motionless, but then liquify when stirred.

Unlike many other liquids, honey has very poor thermal conductivity. Melting crystallized honey can easily result in localized caramelization if the heat source is too hot, or if it is not evenly distributed. However, honey will take substantially longer to liquify when just above the melting point than it will at elevated temperatures.

Since honey contains electrolytes, in the form of acids and minerals, it exhibits varying degrees of electrical conductivity. Measurements of the electrical conductivity are used to determine the quality of honey in terms of ash content.
Variations in the water content alter the refractive index of honey. Water content can easily be measured with a refractometer. Typically, the refractive index for honey will range from 1.504 at 13% humidity, to 1.474 at 25%. Honey also has an effect on polarized light, in that it will rotate the polarization plane. The fructose will give a negative rotation, while the glucose will give a positive one. The overall rotation can be used to measure the ratio of the mixture. Honey has the ability to absorb moisture directly from the air, a phenomenon called hygroscopy. The amount of water the honey will absorb is dependent on the relative humidity of the air. This hygroscopic nature requires that honey be stored in sealed containers to prevent fermentation. Honey will tend to absorb more water in this manner than the individual sugars would allow on their own, which may be due to other ingredients it contains (Root, 2005).

2.1.3. Typical Honey Composition

Honey is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature. It is a sticky and viscous solution with a content of 80–85% carbohydrate (mainly glucose and fructose), 15–17% water, 0.1–0.4% protein, 0.2% ash and minor quantities of amino acids, enzymes and vitamins as well as other substances like phenolic antioxidants. The minor constituents are known to have distinctive nutritional or medicinal properties while the major constituents are nearly the same in all honey samples. The precise chemical composition and physical properties of natural honeys differ according to the plant species on which the bees forage and also according to climatic conditions and vegetations which are important factors that can affect the various properties of honey. The northeast sub-region of Nigeria, which lies within 9°–14°N and 8°–15°E, consists of approximately one quarter of the land mass of Nigeria and comprises of six states (Adamawa, Bauchi, Borno, Gombe, Taraba and Yobe States) characterized by humid, semi-arid and arid climates. The production and marketing of bee products, especially honey is a well known business in the subregion but information about the qualities of the products is scare (Buba et al., 2013). The Standard composition of honey that was recommended by USDA (2012), was also summarized in table (2.1)
Table (2.1) Nutritional value of honey (per 100 g (3.5 oz))

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</tr>
<tr>
<td>- Sugars</td>
<td>82.12 g</td>
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<tr>
<td>- Dietary fiber</td>
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<td>Fat</td>
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<tr>
<td>Protein</td>
<td>0.3 g</td>
</tr>
<tr>
<td>Water</td>
<td>17.10 g</td>
</tr>
<tr>
<td>Riboflavin (vit. B&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>0.038 mg (3%)</td>
</tr>
<tr>
<td>Niacin (vit. B&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>0.121 mg (1%)</td>
</tr>
<tr>
<td>Pantothenic acid (B&lt;sub&gt;5&lt;/sub&gt;)</td>
<td>0.068 mg (1%)</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>0.024 mg (2%)</td>
</tr>
<tr>
<td>Folate (vit. B&lt;sub&gt;9&lt;/sub&gt;)</td>
<td>2 μg (1%)</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.5 mg (1%)</td>
</tr>
<tr>
<td>Calcium</td>
<td>6 mg (1%)</td>
</tr>
<tr>
<td>Iron</td>
<td>0.42 mg (3%)</td>
</tr>
<tr>
<td>Magnesium</td>
<td>2 mg (1%)</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>4 mg (1%)</td>
</tr>
<tr>
<td>Potassium</td>
<td>52 mg (1%)</td>
</tr>
<tr>
<td>Sodium</td>
<td>4 mg (0.5%)</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.22 mg (2%)</td>
</tr>
</tbody>
</table>

Source from, USDA (2012)
2.1.4. Indicators of Quality:

High-quality honey can be distinguished by fragrance, taste, and consistency. Ripe, freshly collected, high-quality honey at 20 °C (68 °F) should flow from a knife in a straight stream, without breaking into separate drops. After falling down, the honey should form a bead. The honey, when poured, should form small, temporary layers that disappear fairly quickly, indicating high viscosity. If not, it indicates excessive water content (over 20%) of the product. Honey with excessive water content is not suitable for long-term preservation.

In jars, fresh honey should appear as a pure, consistent fluid, and should not set in layers. Within a few weeks to a few months of extraction, many varieties of honey crystallize into a cream-colored solid. Some varieties of honey, including tupelo, acacia, and sage, crystallize less regularly. Honey may be heated during bottling at temperatures of 40–49°C (104–120°F) to delay or inhibit crystallization. Overheating is indicated by change in enzyme levels, for instance, diastase activity, which can be determined with the Schade or the Phadebas methods. A fluffy film on the surface of the honey (like a white foam), or marble-colored or white-spotted crystallization on a container’s sides, is formed by air bubbles trapped during the bottling process.

An Italian study determined nuclear magnetic resonance spectroscopy can be used to distinguish between different honeys types, and can be used to pinpoint the area where it was produced. Researchers were able to identify differences in acacia and poly-floral honeys by the differing proportions of fructose and sucrose, as well as differing levels of aromatic amino acids phenylalanine and tyrosine. This ability allows greater ease of selecting compatible stocks (Standifer, 2007).
2.1.5. Nutrition

Honey is a mixture of sugars and other compounds. With respect to carbohydrates, honey is mainly fructose (about 38.5%) and glucose (about 31.0%) making it similar to the synthetically produced inverted sugar syrup, which is approximately 48% fructose, 47% glucose, and 5% sucrose. Honey's remaining carbohydrates include maltose, sucrose, and other complex carbohydrates. As with all nutritive sweeteners, honey is mostly sugars and contains only trace amounts of vitamins or minerals. Honey also contains tiny amounts of several compounds thought to function as antioxidants, including chrysin, pinobanksin, vitamin C, catalase, and pinocembrin. The specific composition of any batch of honey depends on the flowers available to the bees that produced the honey (Martos et al., 2000). Mohammed et al., (2013) reviewed the available and the important of mineral contents in the honey.

2.1.6. Reservation

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 decades and even 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. Honey sealed in honeycomb cells by the bees is considered to be the ideal form for preservation.

Honey should also be protected from oxidation and temperature degradation. It generally should not be preserved in metal containers because the acids in the honey may promote oxidation of the vessel. Traditionally, honey was stored in ceramic or wooden containers; however, glass and plastic are now the favored materials. Honey stored in wooden containers may be discolored or take on flavors imparted from the vessel. Likewise, honey stored uncovered near other foods may absorb other smells.

Excessive heat can have detrimental effects on the nutritional value of honey. Heating up to 37 °C (98.6 °F) causes loss of nearly 200 components, some of which are antibacterial. Heating up to 40 °C (104 °F) destroys invertase, an important enzyme. At 50 °C (122 °F), the honey sugars caramelize. Generally, any large temperature fluctuation
causes decay. Regardless of preservation, honey may crystallize over time. Crystallization does not affect the flavor, quality or nutritional content of the honey, though it does affect color and texture. The rate is a function of storage temperature, availability of "seed" crystals and the specific mix of sugars and trace compounds in the honey. Tupelo and acacia honeys, for example, are exceptionally slow to crystallize, while goldenrod will often crystallize still in the comb. Most honeys crystallize fastest between about 50 and 70 °F (10 and 21 °C). The crystals can be dissolved by heating the honey (Daniel et al., 2009).

2.1.7. Uses of Honey

More than 1,400 years ago ALLAH and His messenger (peace be upon him), told us that honey can heal a variety of medical problems. Traditional uses of honey have included honey mixed with lemon for sore throats and it has also been used for stomach pains and problems, as well as certain eye conditions.

2.1.7.1. In Medicine

Historically, honey has been used by humans to treat a variety of ailments through topical application, but only recently have the antiseptic and antibacterial properties of honey been chemically explained.

In Ayurveda, a 4000-year-old medicine originating from India, honey is considered to positively affect all three primitive material imbalances of the body. It has sweetness with added astringent as end taste. It is heavy, dry and cold. It promotes the healing process." Some wound gels which contain antibacterial raw honey and have regulatory approval are now available to help treat drug-resistant strains of bacteria (MRSA). One New Zealand researcher says a particular type of honey (Manuka honey) may be useful in treating MRSA infections (Knox, 2004).

As an antimicrobial agent honey may have the potential for treating a variety of ailments. Antibacterial properties of honey are the result of the low water activity causing osmosis, chelation of free Iron, its slow release of hydrogen peroxide. High acidity and the antibacterial activity of methylglyoxal.

Honey appears to be effective in killing drug-resistant biofilms which are implicated in chronic rhinosinusitis (Wahdan, 1998).
CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials

3.1.1 Honey samples

Three samples of honey were obtained from Wad Medani local market. The samples included natural honey of bees (HB), industrial black honey (BH) (Trade name: El Bawadi, Ingredients: 100% Sugar and Molasses, Made in Egypt, Production: February 2013) and white honey (WH) (Trade name: Lyle's Golden Syrup, 454 g, Manufactured in United Kingdom, Production: May 2012).

3.2. Methods

3.2.1. Physicochemical characteristics

3.2.1.1. Refractive Index (David, p. (1970)

Determination of the refractive index of the honey samples was done using a refractometer (Appe 60) at a constant temperature of 20 °C.

3.2.1.2. Density (David, p. (1970)

Determination of the weight of the honey per ml was done by using Pyknometer (Appe 60) usually at 20 °C.

3.2.1.3. Total soluble solids (TSS)

Determination of the total soluble solids (TSS) of honey samples was done by using Refractometer ( Appe 60) at a constant temperature of 20 °C

3.2.1.4. Viscosity

Determination by (U shape viscometer) was done by determine density of the water and density of the sample and determine the time it using for cut the distance.

3.2.2. Approximate Chemical Analysis of Honey:

3.2.2.1 Moisture content

Moisture content of honey was determined according to the (AOAC, 1990). An empty open dish was heated for 30 minutes in an oven at 105°C, then removed from the oven, covered with a lid and placed in desiccators at room temperature then weighted. 5 g of the sample were placed in the dish and weighed with the lid on it, and then placed in
the vacuum oven for three hours at 105°C. The dish with lid were removed from desiccators and weighed at room temperature. 

Moisture content percentage was calculated as:

\[
\text{Moisture content (\%) } = \frac{W_2 - W_3}{W_2 - W_1} \times 100
\]

Where:

- \( W_1 \) = weight (g) of empty dish.
- \( W_2 \) = weight (g) of dish plus sample before drying.
- \( W_3 \) = weight (g) of dish plus sample after drying.

**3.2.2.2. Ash content**

The ash content was determined according to (AOAC, 1984). Two grams of sample were weighed on dry basis and ignited in Heraeues electronic muffle furnace at 550°C for 5 hours. The ash content was then calculated as percentage as follows:

\[
\text{Ash (\%) } = \frac{W_3 - W_1}{W_2 - W_1} \times 100\%
\]

Where:

- \( W_1 \) = weight of the empty crucible
- \( W_2 \) = weight of the crucible plus the sample
- \( W_3 \) = weight of the crucible plus Ash

**3.2.2.3. Nitrogen and protein content**

The kjeldahl method was used to determine the amount of nitrogen of the different samples according to (AOAC, 1984) then multiplied by a factor of 6.25. The method was composed of three major steps:

- **a-** The digestion: About 1 g of the honey sample in addition to 10 ml of the \( \text{H}_2\text{SO}_4 \) were put in 500 ml beaker for one hour for digestion
- **b-** The distillation: The tube was placed in the distillation equipment and 30 ml 40% \( \text{NaOH} \) solution was added. The ammonia was distilled into 20 ml boric acid indicator solution.
- **C-** The titration: The mixture then titrated with 0.05 N HCl (colour: green-purple). The protein content was then calculated as follows:

\[
%N = \frac{V \times T \times 14 \times \text{Volume of diluent digest}}{\text{Volume of diluent digest}} \times 100
\]
\[ 1000 \times G \]

\[ \% \text{ protein} = \% \text{ N} \times 6.25 \]

Where:

- \( N \): nitrogen content.
- \( V \): volume (ml) of the HCl solution
- \( T \): normality of HCl.
- \( G \): weight (g) of the sample.

### 3.2.2.4. pH Measurement

About 10 g of sample was dissolved in 75 ml of carbon dioxide – free water in 250 ml beaker, stirred with a magnetic stirrer. The electrodes of the pH meter (PHS-3C Digital) were then immersed in the solution and the pH was read.

### 3.2.2.5. Mineral content

The mineral content percentage was determined according to (AOAC, 1984). Two gram of sample were weighed on dry basis and ignited in Heraeues electronic muffle furnace (Acorden 400, made in England) at 550°C for 5 hours. The ash content was then calculated as percentage as follows:

\[
\text{Ash (\%) = } \frac{W_3 - W_1}{W_2 - W_1} \times 100\%
\]

Where:

- \( W_1 \): weight of the empty crucible
- \( W_2 \): weight of the crucible plus the sample
- \( W_3 \): weight of the crucible plus Ash

The ash was then moistened with water. 5 ml of 33% HNO₃ were added together with the 10 ml of HCL then the mixture was evaporated to dryness on a water-bath. A blank determination was carried out. The solution was used to determine (Ca, Fe, Mn, P, Na, K, Cl, and Zn). The minerals were detected automatically using atomic absorption device (Phoemix 986, made in England).

### 3.2.2.6 Energy (Oshea, A. and Magwire, A. (1962)).

Reagents

1.5 N potassium dichromate solution
About 73.54 g of potassium dichromate were dissolved in 100 ml of warm distilled water and the volume was completed to 1 litre.

**0.15 N sodium thio-sulphate solution**

About 37.23 g of sodium thio-sulphate were dissolved in 100 ml of warm distilled water and the volume was completed to 1 litre.

**Potassium iodide sodium bicarbonate solution:**

About 100 g of potassium iodide and 32 g of sodium bicarbonate were dissolved in distilled water and diluted to 500 ml. It is prepared fresh every time.

**Conc. sulphuric acid.**

**Procedure**

One gram of each sample was oxidized with 20 ml of 1.5 N potassium dichromate solution and with 40 ml of concentrated sulphuric acid for 90 minutes. The volume was completed to 250 ml with distilled water. To a 2.5 ml aliquot, 10 ml of potassium iodide sodium bicarbonate solution was added in a dark (to avoid exposure to light) for 25 minutes. The contents were diluted with 50 ml distilled water and the liberated iodide was titrated against 0.15N sodium thio-sulphate using starch as an indicator to light green end point. The duplicate blanks were also run for each set, the amount of 1.5N potassium dichromate used for oxidizing the sample was calculated by subtracting the above reading from the blank (C). The energy value was calculated by following equation:

Energy (Kcal/100g) =

\[
\frac{\text{ml of 1.5 N potassium dichromate used for Oxidizing 1 g of sample x 100}}{\text{Oxidizing coefficient}}
\]

Oxidizing coefficient =23.39 - 0.069 p + 0.00026 p²

Where, p =true protein

**3.2.2.7. Carbohydrates**

**3.2.2.7.1 Glucose standard**

**Procedure**

Two gram glucose were weighed, then dissolved in 100 ml distilled water, and 10 ml were taken and diluted in 1000 ml distilled water then put in tubes 0.1, 0.2, 0.3, 0.4, and 0.5 ml, then the volume was completed in each tube to 1.0 ml by distilled water then
1.0 ml copper reagent in addition to 1.0 ml hardening reagent were added, heated for 10 minutes, cooled and 1.0 ml Nelson reagent and 6.0 ml distilled water were added. The spectrometer in 600 wave length was used to take reading and the curve was then made.

**3.2.2.7.2. The Reducing Sugar (RS)**

**Procedure**

Two gram of sample plus 50 ml distilled water were added and shaken for ½ hour. One spoon of charcoal was also added and then filtrate. Analysis: about 0.1 ml (or less) mixture was taken and 1.0 ml copper reagent plus 1.0 ml hardening reagent were added, heated for 10 minutes then cooled and 1.0 ml Nelson reagent plus 6.0 ml distilled water were added. The spectrometer in 600 wave length was used for calculation as follow:-

\[
\text{R.S} = \frac{X}{0.1} \times \frac{50}{2} \times \frac{100}{1000} \times 1000
\]

\[
\text{R.S} = \frac{X}{40}
\]

X = concentration of reducing sugar in sample.

**3.2.2.7.3. Total soluble sugars (TSSG)**

About 20 ml of the above extract, plus 20 ml oxalic acid (1% N) were added, heated for ½ hour, refluxing with (2N) NaOH then the volume was completed to 50 ml by distilled water.

**Analysis**

Take 0.5 ml (or less) then 1.0 ml copper reagent plus 1.0 ml hardening reagent were added, heated for 10 minutes then cooled and 1.0 ml Nelson reagent in addition to 6.0 ml distilled water were added. The spectrometer in 600 wave length was used.

**Calculation**

\[
\text{Sucrose} = \frac{X}{0.5} \times \frac{50}{2} \times \frac{50}{20} \times \frac{100}{1000} \times 1000
\]

\[
\text{Sucrose} = \frac{X}{80}
\]

X = concentration of total soluble sugars in sample.

**3.3. Statistical analysis**

The obtained data were subjected to histogram and Pie chart, simple descriptive statistics and ANOVA two factors using Excel-2007 Program.
CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Physicochemical characteristics of honey

The density, refractive index, viscosity and pH of three samples of honey (honey of bees (HB), black honey (BH) and white honey (WH)) were presented in Table (4.1). The density were 1.40, 1.35 and 1.37, respectively for HB, BH and WH samples. The same order showed refractive index of 1.501, 1.483 and 1.497, viscosity (poise) at 25°C of 521, 209 and 302 and pH of 4.5, 5.2 and 5.6.

It was clear that, natural honey (HB) had a higher density and refractive index and a medium viscosity and lowest pH values, while that, the industrial white honey (WH), had highest viscosity and pH, median refractive index and density values. The Black honey (BH), had a median refractive index and pH, and lowest density and viscosity in comparison to HB and WH.

The viscosity of honey is affected greatly by both temperature and water content. The higher the humidity, the easier honey will flow. Above its melting point, however, water has little effect on viscosity. A side from water content, the composition of honey also has little effect on viscosity, with the exception of a few types. At 25 °C (77 °F), honey with 14% humidity will generally have a viscosity of around 400 poise, while a honey containing 20% humidity will have a viscosity of around 20 poise. Viscosity increase due to temperature occurs very slowly at first (Root, 2005).

The standard pH value for honey, according to the USA standard is 3.96 (not in range, 1977), which was relatively closer to the natural honey of bee (HB) and less than those of WH and BH.

The standard refractive index for honey range from 1.504 at 13% humidity, to 1.474 at 25% (Root, 2005). The obtained values of refractive index for the three types of honey were within that range.
Table (4.1): The density (g/cm³), refractive index (degree), viscosity (poise) and pH characteristics of three samples of honey

<table>
<thead>
<tr>
<th>Sample</th>
<th>DENSITY (g/cm³)</th>
<th>Refractive index (degree)</th>
<th>Viscosity (poise)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB</td>
<td>1.40</td>
<td>1.501</td>
<td>302</td>
<td>4.5</td>
</tr>
<tr>
<td>BH</td>
<td>1.35</td>
<td>1.483</td>
<td>209</td>
<td>5.2</td>
</tr>
<tr>
<td>WH</td>
<td>1.37</td>
<td>1.497</td>
<td>521</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Simple Descriptive Statistics

<table>
<thead>
<tr>
<th></th>
<th>Sum</th>
<th>Mean</th>
<th>SE</th>
<th>Max</th>
<th>Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>DENSITY (g/cm³)</td>
<td>4.12</td>
<td>1.37</td>
<td>0.02</td>
<td>1.40</td>
<td>1.35</td>
</tr>
<tr>
<td>Refractive index (degree)</td>
<td>4.48</td>
<td>1.49</td>
<td>0.005</td>
<td>1.501</td>
<td>1.483</td>
</tr>
<tr>
<td>Viscosity (poise)</td>
<td>1032</td>
<td>344</td>
<td>92.48243</td>
<td>521</td>
<td>209</td>
</tr>
<tr>
<td>pH</td>
<td>15.30</td>
<td>5.10</td>
<td>0.32</td>
<td>5.60</td>
<td>4.50</td>
</tr>
</tbody>
</table>

HB: natural honey of bees
BH: commercial black honey
WH: commercial white honey
Figure (4.1): The density, refractive index, viscosity and pH characteristics of three samples of honey

HB: natural honey of bees
BH: commercial black honey
H: commercial white honey
4.2. Some chemical characteristics of honey and energy

The results of analysis of some chemical characteristics (moisture, ash, protein and energy contents) were presented in (Table 4.2) and (Fig. 4.2). The moisture content of honey of bees (HB), commercial black honey (BH) and commercial white honey (WH) samples were 25.5%, 35.0%, and 26.4%. These values were more than the USA Standards for honey which is 17.2% (David, 1970).

The highest moisture content was that of BH which was 35% and the lowest was that of HB which was 25.5%, while the value of WH was 26.4 %. The obtained values of the moisture contents indicated excessive water content (over 20%) for all tested samples. In similar work conducted by Mahmoud (2012), the moisture content in 10 samples of honey ranged between 10.24 – 36.87%, which reflected a wide range of variation, whether for a natural reasons (climatic) or as a result of cheating.

The different samples of honey had relatively similar ash content which were ranged between 0.5 - 1.5%. These values are also more than that of Buba et al., (2013) which was 0.2%. Mahmoud ( 2012, found a range of 0.5 – 2.0% for the ash content in his work that involving 10 honey samples.

The protein content of honey samples were not highly varied (the highest value was of BH (0.344), and the lowest was of WH (0.175), while HB was 0.203 %. Buba et al., (2013) found that, the range of protein in honey was 0.1–0.4%. The contents of protein in this study were 0.17- 0.34%. The standard of quality was 0.3 % (USDA, 2012). Unlike Mahmoud (2012), who found that, the protein contents in 10 honey samples ranged between 4.37 – 6.01%, which indicate cheating in that samples.

The energy in the honey samples were 39.78, 41.48 and 31.23 Kcal/100g, respectively, for HB, BH and WH. The richest product with energy was the black honey, followed by the natural honey of bees and lastly the industrial white honey (Table, 4.2 and Fig, 4.2). The main factor that determining the honey content of calories is its nutritional composition of energy units (main nutrients; dissolved carbohydrates, proteins and lipids). It was clear that, BH possessed more energy units than HB and WH.
Table (4.2) Some chemical characteristics: moisture, ash, protein and energy contents of the three types of honey

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
<th>Protein (%)</th>
<th>Energy(Kcal/100g)</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB</td>
<td>25.50</td>
<td>0.50</td>
<td>0.20</td>
<td>39.78</td>
<td>34.02</td>
</tr>
<tr>
<td>BH</td>
<td>35.00</td>
<td>1.50</td>
<td>0.34</td>
<td>41.48</td>
<td>21.68</td>
</tr>
<tr>
<td>WH</td>
<td>26.40</td>
<td>1.00</td>
<td>0.17</td>
<td>31.23</td>
<td>41.2</td>
</tr>
</tbody>
</table>

Simple Descriptive Statistics

<table>
<thead>
<tr>
<th></th>
<th>Sum</th>
<th>3</th>
<th>0.72</th>
<th>112.49</th>
<th>96.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>29</td>
<td>1</td>
<td>0.24</td>
<td>37.5</td>
<td>32.3</td>
</tr>
<tr>
<td>SE</td>
<td>3.03</td>
<td>0.29</td>
<td>0.05</td>
<td>3.17</td>
<td>5.70</td>
</tr>
<tr>
<td>Max</td>
<td>35</td>
<td>1.5</td>
<td>0.344</td>
<td>41.48</td>
<td>41.2</td>
</tr>
<tr>
<td>Min</td>
<td>25.5</td>
<td>0.5</td>
<td>0.175</td>
<td>31.23</td>
<td>21.68</td>
</tr>
</tbody>
</table>

HB= honey of bees
BH= black honey
WH= white honey
Figure (4.2) The Pie chart for some chemical characteristics: moisture, ash, protein and energy contents of the three types of honey (HB: natural honey of bees, BH: commercial black honey and WH: commercial white honey)
4.3 Minerals Content of Honey

Table (4.3) and Fig (4.3), showed the percentage of Cu that ranged between 0.12% - 0.16%, Na ranged between 0.52% - 0.65%, K ranged between 1.20% - 1.60%, Fe ranged between 0.70% - 0.81%, Zn ranged between 0.07% - 0.09%, Mn ranged between 0.06% - 0.09%. Ca ranged between 0.23% - 0.30% and P ranged between 0.13% - 0.16%. Honey is richer in minerals that render it unsuitable for winter stores. This result were very close to that of Mahmoud (2012), in which Na ranged between 0.54% - 0.70%, K ranged between 1.2% - 2.3%, Fe ranged between 0.70% - 0.87%, Zn ranged between 0.089% - 0.105%, Mn ranged between 0.08% - 0.099%. Ca ranged between 0.20% - 35% and P ranged between 0.10% - 0.20%.

The amount of minerals in this study were relatively similar to that of USDA (2012) standards in both K and Na, whereas, the three samples were less in their Ca and P contents (standard= 1%), less in their Zn contents (standard= 2%) and less in their Fe contents (standard= 3%)

ANOVA analysis revealed that, there were significant differences in the rows level (honey samples; f-stat= 3.69, f-cit= 2.73) and in the columns level (types of mineral; f-stat= 123.21, f-cit= 2.19), i.e. the three types of honey were not similar in their mineral contents, also the quantities of mineral contents were at considerable variation.

The mineral contents in natural honey (HB) observed to be more than that of commercial white honey (WH) and (BH). This finding agreed with the suggestion of Truchado et al. (2011), who stated that: the darker color the honey, the higher its mineral contents, the pH readings, and the aroma/flavor levels. Minerals such as potassium, chlorine, sulfur, iron, manganese, magnesium, and sodium have been found to be much higher in darker honeys, but the commercial black honey (BH), was not so, and this may simply referred to its commercial origin.

Honey color graded into light, amber, and dark categories which do not really have any bearing on quality. Some of the most distinctively and strongly flavored honey varieties, such as basswood, are very light, while very mild and pleasant honeys such as tulip poplar can be quite dark. While it is not an indicator of honey quality and there are exceptions to the rule, generally speaking (Truchado et al., 2011).
Table (4.3): The percentages of minerals content (Cu, Na, K, Fe, Zn, Mn, Ca, P)% in the three types of honey

<table>
<thead>
<tr>
<th>Content</th>
<th>Cu</th>
<th>Na</th>
<th>K</th>
<th>Fe</th>
<th>Zn</th>
<th>Mn</th>
<th>Ca</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB</td>
<td>0.16</td>
<td>0.65</td>
<td>1.6</td>
<td>0.81</td>
<td>0.09</td>
<td>0.09</td>
<td>0.30</td>
<td>0.16</td>
</tr>
<tr>
<td>BH</td>
<td>0.12</td>
<td>0.64</td>
<td>1.2</td>
<td>0.70</td>
<td>0.07</td>
<td>0.06</td>
<td>0.23</td>
<td>0.13</td>
</tr>
<tr>
<td>WH</td>
<td>0.14</td>
<td>0.52</td>
<td>1.3</td>
<td>0.74</td>
<td>0.08</td>
<td>0.08</td>
<td>0.24</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Simple Descriptive Statistics

<table>
<thead>
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HB= honey bees
BH= black honey
WH= white honey

ANOVA

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Figure (4.3): The percentage of some minerals contents of the three types of honey (HB: natural honey of bees, BH: commercial black honey and WH: commercial white honey)
4.4 Carbohydrates of Honey

The highest percentage (58%) of reducing sugar (R.S.) was found in the WH, followed by HB (56%), while the lowest value (22.4%) was in the BH.

The percentage non R.S the value was higher in BH sample (28.6%), and it was (16%) in HB, while it was (14%) in WH sample.

Total soluble sugars percentage was similar in HB and WH samples (72%), while that of BH was just 51%. The standard glycemic index ranges from 31 to 78, depending on the variety (David, 1970). Accordingly, TSS content of the three types are within that recommended range (Table, 4.4 and Fig, 4.4).

Honey is a mixture of sugars and other compounds. With respect to carbohydrates, honey is mainly fructose and glucose making it similar to the synthetically produced inverted sugar syrup, which is approximately 48% fructose, 47% glucose, and 5% sucrose. Honey's remaining carbohydrates include maltose, sucrose, and other complex carbohydrates. As with all nutritive sweeteners, honey is mostly sugars and contains only trace amounts of vitamins or minerals. Honey also contains tiny amounts of several compounds thought to function as antioxidants, including chrysin, pinobanksin, and vitamin C. The specific composition of any batch of honey depends on the flowers available to the bees that produced the honey (Martos et al., 2000).

The percentage total soluble solids (TSS) were also varied among the honey samples. The value was higher in HB sample (74.8%), and it was 65.0% in the BH, while it was 70.6% in the WH sample (Table, 4.4 and Fig, 4.4). The obtained values of total soluble solids (TSS) in this work were within the range obtained by Mahmoud (2013), who found that, the TSS in 10 samples of honey ranged between 62.23 – 82.86 % in 9 samples, while the 10th sample have TSS of 38.35%.

Honey is an easily digestible foodstuff containing a range of saccharides, organic and amino acids, mineral matter, colours, aromatic substances and a trace amount of fats (Redtke and Hadtke, 1998; Bogdanov et al., 1998). Beside these honeys contains very valuable but unstable compounds, such as enzymes, substances of hormonal character, some vitamins and a few minor compounds (Yilmaz and Yavuz 1999; Qiu et al. 1999).
Table (4.4): The total soluble sugars (TSSG), reducing sugars (R.S.) and non reducing sugars and total soluble solids (TSSD) in the three types of honey

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total soluble sugars (TSSG %)</th>
<th>Reducing sugar % (R.S.)</th>
<th>Non R.S</th>
<th>Total soluble solid % (TSSD)</th>
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<tbody>
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<td>HB</td>
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<td>56</td>
<td>16</td>
<td>74.8</td>
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<tr>
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<td>22.4</td>
<td>28.6</td>
<td>65</td>
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<tr>
<td>WH</td>
<td>72</td>
<td>58</td>
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<td>70.6</td>
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Simple descriptive Statistics

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HB= honey bees
BH= black honey
WH= white honey
Figure (4.4) The percentage total soluble sugars (TSSG), reducing sugars (RS) and percentage total soluble solids (TSSD) of the three types of honey (HB: natural honey of bees, BH: commercial black honey and WH: commercial white honey)
CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

1- The standard pH value for honey was relatively closer to that of natural honey of bee (HB) and less than those of white honey (WH) and black honey (BH), while that the obtained values of refractive index for the three types of honey were within the range of honey standards.

2- The moisture content of HB, BH and WH samples were more than the USA Standards, while the different samples of honey had relatively similar ash content. The contents of protein although it was less than 0.35% in best case but it still within the standards of quality.

3- Total soluble sugar (TSS) content of the three types of honey is within the recommended range.

4- The mineral contents in HB observed to be more than that of WH and BH.

5- The richest product with energy was the BH, followed by the HB and lastly the WH.

6- The highest percentage of soluble sugar (SS) was found in the WH, followed by HB, while the lowest value was in the BH.

7- The percentage total soluble solids (TSS) were higher in HB sample, followed by WH and lastly BH sample.

5.2. Recommendations

1- The imported commerce honey should be tested at the boarders with reference to the quality standards of the honey.

2- The factors affecting the honey properties and quality should be determined.
References


