BIOCHEMICAL CHARACTERIZATION OF 'KELULUT' (*TRIGONA* SPP) HONEY

by

NOR ADLIN BT MD YUSOFF

Dissertation submitted in partial fulfilment of the requirements for the degree of Bachelor of Health Sciences (Biomedicine)

March 2005

CERTIFICATE

This is to certify that the dissertation entitled "Biochemical Characterization of 'Kelulut' (*Trigona* spp) Honey"

is the bonafide record of research work done by

Miss Nor Adlin Bt. Md Yusoff

During the period of **October 2004** to **March 2005** under our supervisions

Signature of Supervisor:

Shahjahan

Dr Mohd. Shahjahan,
School of Health Sciences,
Universiti Sains Malaysia,
Health Campus,
16150 Kubang Kerian,
Kelantan.

Date: 4th April. 2005

Signature of Co Supervisors:

Prof Syed Mohsin Syed Sahil Jamalullail
School of Health Sciences,
Universiti Sains Malaysia,
Health Campus,
16150 Kubang Kerian
Kelantan.

Date: 04/04/05

Dr Shaharum Shamsuddin
School of Health Sciences,
Universiti Sains Malaysia,
Health Campus,
16150 Kubang Kerian,
Kelantan.

Date: 04/04/05

ACKNOWLEDGEMENT

Bismillahirrahmanirrahim. In the name of Allah Taala The Most Gracious, Most Merciful. Shalawat and rememberance for the Holy Prophet, Muhammad s.a.w.

Alhamdullilah, with the God's help and His amazing grace, I'm finally completed this dissertation after going through the six months of challenging and stressful period. Nevertheless, this period is so meaningful because it gives me an opportunity to step foot into the realm of the true research work. This research has been developed and implemented with assistance of several generous individuals. I would like to take this opportunity to express my appreciation to their efforts and kindness.

First and foremost, I thank my supervisor, Dr Mohd. Shahjahan for his continuous support in the research project. He shows me different ways to approach a research problem and the need to be persistent to accomplish any goal. I also thank both of my co-supervisors, Prof Syed Mohsin Syed Sahil Jamalullail and Dr Shaharum Shamsuddin. Their achievements are my inspiration.

I would like to extend my thankfully gratitude to Dr Noor Izani Noor Jamil as a Coordinator for final year research project. He taught me how to write a thesis in a

well organised way. Without his encouragement and constant guidance, I could not have finished this dissertation.

A special thanks goes to Dr. Wan Rosli Wan Ishak, who is most responsible for helping me during my works at Dietetic Laboratory. He had taught me the important for following the test procedure accurately in preventing any incident during the experiment and still gave me a chance even though I had done a big mistake. Also thanks to the technologists there especially Kak Jee who was always comforting me and had a confidence in me when I doubted myself.

I was also greatly indebted to Puan Nor Azizah Abdullah, Scientific Officer of School of Health Sciences who dedicated her precious time in helping me. She was always there to help me in solving any unsolvable problems concerning my research project especially regarding my laboratory work. Also thanks to the staffs at the Laboratory Facility Unit (UKM): Cik Jastina, Puan Nor Azlin, En. Nik Fakharuddin, En. Amri, En. Azwan, En. Azilah and other staffs for interesting discussion and offering help in providing the materials and sometimes assisting me during my laboratory works.

Thanks also go to Puan Herlina Patrick for her intention regarding my needs during my works at Biomedicine Laboratory. She made the Biomedicine Laboratory a wonderful and safe workplace under her supervision. Also thanks to the folks at the Biomedicine Laboratory for allowing me to use their apparatus and being fun to be with. Not forgotten, Puan Rosniah and En. Sanusi from Forensic Laboratory for their generous support and help in handling the atomic absorption spectroscopy.

Let me also 'thank you' to the rest of my thesis committee especially Nur Nadzifah and Noorshazilawati who made the weather in the workplace more cheerful and had kept me in good spirits since we were sharing the same workplace, Biomedicine Laboratory. Also thanks to my good friends from Biomedicine course, Murni, Mardiaty Iryani, Norayuni, Nurul Syahmi, Siti Fatihah and other clicks. Thank you for being by my side and for sharing unforgettable moments with me during the last three years. Both of my generous seniors, Puan Norizah and Puan Nurul Arsyiah deserve a special mention. Your previous beautiful dissertations are my main reference. I am truly thankful.

I also like to say a heartfelt 'thank you' to my parents, En. Md Yusoff b Hassan and Puan Sepiah @ Razinah bt Mahmood for giving me life in the first place, for continuous prayers and for unconditional support and encouragement to pursue my interests, even when the interests went beyond their expectations. My elder sisters, Rena Rozaini and Siti Harwani for listening to my complaints and frustrations and for believing in me. My younger brother and sisters for cheering me up.

Last, but not least, to those who are not mentioned here, you are never forgotten, I thank you all.

Nor Adlin.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

amount of sugar was 73.81%. Protein and lipid were detected in a trace amount in the honey sample. The amount of protein was 7.86% and the amount of lipid was only 1 mg/ 100g. Minerals were also present in very small quantities. Among these four determined mineral elements, calcium being the most abundant with the value of 8.07 mg/ 100 g.

ABSTRAK

Madu yang dihasilkan oleh lebah tanpa sengat, *Trigona* spp. dikenali sebagai kelulut di Malaysia. Madu ini merupakan ubatan berharga dalam kalangan masyarakat setempat dan digunakan untuk merawat beberapa jenis penyakit. Madu daripada kelulut dikatakan lebih berkesan berbanding madu yang dihasilkan oleh lebah biasa. Antara kegunaan madu kelulut adalah untuk merawat gangguan sistem penghadaman, batuk, tonsilitis, sakit tekak, ulser perut dan usus, kesejukkan, penyakit pada kawasan mulut dan membran mukus serta sebagai pembalut luka. Data saintifik mengenai madu kelulut di dalam jurnal adalah terhad. Sejajar dengan itu, kajian ini dijalankan bagi menentukan ciri – ciri fizikal dan biokimia madu kelulut dan juga menambah data saintifik berhubung madu kelulut.

Bagi pengkelasan fizikal, ciri – ciri seperti warna, pengkristalan, pH, kespesifikan graviti, kelembapan dan kandungan abu ditentukan. Bagi pengkelasan biokimia, madu kelulut dianalisis bagi menentukan kandungan gula, gula penurun, protein dan lipid. Madu kelulut disediakan melalui teknik pencairan, menggunakan faktor pencairan spesifik berdasarkan kaedah ujian yang digunakan. Kandungan metal yang dikaji adalah kalsium (Ca), ferum (Fe), kuprum (Cu) dan zink (Zn). Elemen – elemen ini dikaji dengan menggunakan 'atomic absorption spectroscopy'.

Keputusan yang diperoleh dibandingkan dengan nilai nutrisi madu berdasarkan klasifikasi USDA. Kandungan gula penurun telah dikesan dalam jumlah yang tinggi iaitu sebanyak 66.86%. Jumlah kandungan gula secara keseluruhannya ialah sebanyak 73.81%.Kandungan protein dan lipid adalah rendah di dalam madu kelulut iaitu sebanyak 7.86% bagi protein dan hanya 1 mg/ 100g bagi lipid. Metal juga dikesan dalam kuantiti sedikit. Antara keempat – empat elemen yang dikaji, kalsium merupakan elemen yang paling tinggi kandungannya di dalam madu dengan nilai sebanyak 8.07 mg/ 100g.

1.0 INTRODUCTION

Honey was the first bee product used by humankind in ancient time. The history of the use of honey is parallel to the history of man and in virtually every culture. Evidence can be found of its use as a food source and as a symbol employed in religions, magic and therapeutic ceremonies (Krell, 1996).

The healing power of honey already mentioned in the Holy Koran (the main reference for Muslims) more than 1400 years ago. This is mentioned in Surah An-Nahl (the Bee), part 16, sentences 68 – 69.

"And thy Lord inspired the bee, saying: Chose thou habitations in the hills and in the trees and in that which they thatch; Then eat all fruits, and follow the ways of thy Lord, made smooth (for thee). There cometh forth from there bellies a drink diverse of hues, wherein is healing for mankind. Lo! Herein is indeed a portent for people who reflect".

The Greeks and Romans called it 'ambrosia' and thought it was the food of the Gods that ensured their immortality. The ancient Egyptian and Sumerian physicians about 4000 years ago used honey to treat disease of the stomach and intestines. Traditional African medicinal extracts are also mixed with honey. In Europe, many traditional formulations involving honey are also known and some were even recommended by Hippocrates, the Father of Modern Medicine (Adams, 1939). The Chinese, Indians and Persians also appreciated its value.

Ibnu Sina, the Prince among Muslim physicians listed several beneficial uses of honey in his monumental work of medicine 'Cannun Fit Tibb (Canon of Medicine)' such as preservation of youthfulness, improvement of memory, trigger feeling of peace and happiness, facilitate digestion, increase in appetite and helps

promote in one's rendering of speech (Khalil *et al.*, 2001). Thus, the significant values of honey can not be denied.

Although the use of honey had been known since the ancient civilization, the actual concept and principle that lies behind the healing activities of honey was only revealed scientifically on the year 1937 (Crane, 1975). Till now the interest on honey continue to gain and develop very well indeed. There is a renewed interest in honey treatment as evidenced by the encouraging number of reports in scientific journal. Nevertheless, most of the study are done on the common honeybee, *Apis* spp. Honey from stingless bees however, that are distributed in the tropics and subtropics is less studied, even though is locally collected and widely used by aborigines (Heard, 2001).

1.1 Honey

Honey is the natural sweet substances 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 comb to ripen and mature. This is the general definition of honey cited from Codex Alimentarius, 1989.

Honey from non – stinging social bee or 'Kelulut' are generally more liquid and vary widely in flavour. The color of this honey also darker than the color of the common honeybee, *Apis* spp honey. Stingless bee honey also has a distinctive bush taste which is a mix of sweet and sour with a hint of lemon. The taste comes from plant resins which the bees use to build their hives and honey pots and varies depending on the flowers and trees visited (Pyper, 2003).

Honey of the stingless bee, *Trigona* spp., is usually a highly praised apitherapeutic agents used as a panacea against different ailments in Ethiopia. Among the most common uses of stingless bee honey are to treat stomach disturbance, cough, tonsillitis, sore throat, stomach and intestinal ulcers, cold, disease of the mouth, mucus membrane, and as a wound dressing (Garedew *et al.*, 2003).

'Kelulut' honeys, often different from species to species, have a much higher water content, are more acidic and have a stronger bacteriostatic (inhibitory) effect than *A. mellifera* honey and contain no diastase (Krell, 1996). Due to the very low amount of honey produced by a colony at one time and the presumed reputation of healing power, the stingless honey price is higher compared to the other types of honey.

Fig. 1.1: 'Kelulut' honey

1.2 'Kelulut'

Stingless bees (family: Meliponinae), like honeybees (family: Apinae), are eusocial insects. Meliponines are eusocial and live in colonies of a few hundred to several thousand workers (Sakagami, 1982). Taxonomically, species of stingless bees belong to the family of Meliponinae and the genera Melipona and Trigona or 'Kelulut'. 'Kelulut' or stingless bees are a group of exclusively tropical social bees which has only vestigial stings, but defend their colonies in other-equally effective ways (Azura, 1995). They are mainly met in the tropical and subtropical regions. Some stingless bees are the smallest bee ever known.

Stingless bees, in a general way, build more complex nests than *Apis mellifera* nests with a great variety of forms, size and place of construction. They construct small nests in tree cavities or on branches, in termite and ant nests, under ground, or in artificial cavities, like walls and tombs in cemeteries (Wille, 1979; Michener, 1974). A total of 275 nests of 12 species of stinglees bees were located in lowland dipterocarp forests in Sabah, Malaysia. Eltz *et al.* (2001) had reported that all nests were closely associated with living (91.5%) and dead (8.5%) trees, either within pre-formed cavities in the trunk (cavity nests) or situated in or under the tree base (base nests).

Stingless bees proved to be generalist foragers that used a large range of plant species as pollen sources. Stingless bees also collect nectar, which they store in an extension of their gut called a crop. Back at the hive, the bees ripen or dehydrate the nectar droplets by spinning them inside their mouthparts until honey is formed. This process concentrated the nectar and increases the sugar content from between 20 and 40% to about 80%. The bees store the pollen and honey in large egg-shaped pots made of cerumen which consists of beeswax mixed with a

plant resin called propolis. These pots are irregularly arranged around the central brood comb. Unlike a hive of honey bees which can produce 75 kg of honey a year, a hive of stingless bees produce less than 1 kg per year.

Beekeeping of stingless bees is emerging in Australia, but there is no beekeeping of stingless bees in Malaysia. Most of the stingless bees honey is collected from the wild stingless bees. The purpose of stinglessbeekeeping is mainly for crop pollination (Arsyiah *et al.*, 2004). Meliponines are among the most predominant flower-visiting insects in the canopy and understory of Asian tropical forests (Inoue *et al.,* 1990; Momose *et al.,* 1998) probably providing important pollinator services during both general and non-general flowering seasons (Momose *et al.,* 1998).

Fig. 1.2: Stingless bee, *Trigona* spp.

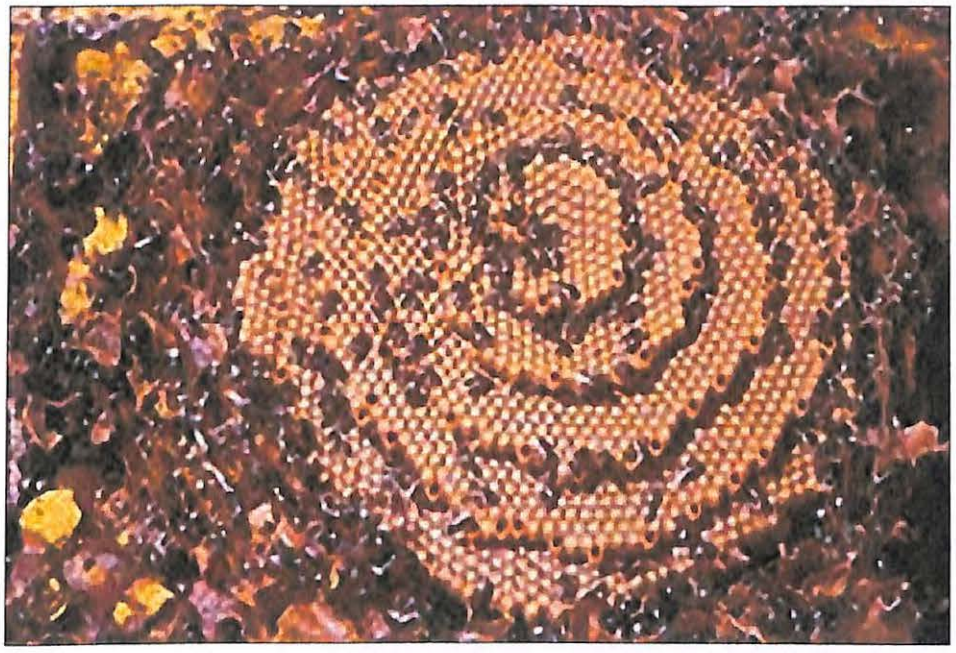


Fig. 1.3: The pattern of stingless bee's hive.

1.3 Objectives

The aims of the present study were

1. To elucidate the physical and biochemical characteristics of 'Kelulut' honey.
2. To prove scientifically the medication values of 'Kelulut' honey.
3. To introduce 'Kelulut' honey as one of the potential remedies in clinical treatments.

2.0 LITERATURE REVIEW

Honey is a natural food, rich in essential nutrients, produced by different species of bees. The medicinal properties of honey have been known since ancient times. For many years, honey attracts scientists because of its natural power healing. Till now the interest towards honey gains and develops very well indeed. As a result, there is a renewed interest in honey treatment as evidenced by the number of reports appearing in the scientific journal.

Honey contains sugars as a source of energy, amino acids, minerals and vitamins which were shown to enhance cell proliferation and hydroxyproline synthesis in the newly formed granulation tissues (A.M. Aljadi and M.Y. Kamaruddin, 2001; White J.W., 1975). The major sugars present are glucose and fructose followed by a lower concentration of sucrose and maltose (Siddiqui and Furgula, 1976). Glucose is essential to continue the supply of energy for the activities of cardiac muscle. Honey which is contains readily absorbable glucose are the main cause of useful effect of honey on heart.

A study conducted by Bogdanov *et al.*, (1998) had reported that besides a high content of a range of saccharides, there are also organic acids, amino acids, mineral matter, colors, aromatic substances and a trace amount of fats. Among analysed minerals, concentration of K, Ca, Mg, Fe and P in native bee were found to be higher than those in foreign bee honey (Bok *et al.*, 1983). It also contains vitamins such as vitamin B_1, B_2, C and nicotinic acid. About 18 organic acids have been detected in honey. The mineral content and trace elements in honey samples could give an indication of the geographical origin of honey (Rodriquez-Otero and Paserio, 1992).

The composition and properties of honey varies with flora utilized by the bees as well as regional and climatic conditions (Trstenjak *et al.,* 1993; Salinas *et al.,* 1994; Perez Arquillue *et al.*, 1994). There are also differences in chemical conditions which are reflected in many physiochemical properties, such as in the content of ash, the spectrum of saccharides, the activity of enzymes, electrical conductivity, pH and optical rotation (Bogdanov *et al.*, 1987; Sanjuan *et al.*, 1997; Golob and Plestenjak, 1999).

Frequently, claims are voiced that honey is good for diabetics (Krell, 1996). Honey also used in pharmaceutical preparations applied directly on open wounds, sores, ulcers and burns. It helps against infections, promotes tissue regeneration and reduces scarring also in its pure, unprocessed form (Krell, 1996). Clinical cases or traditional claims that honey reduces and cures eye cataracts (Mikhailov, 1950), normalizes kidney function, reduces fever, helps insomnia and improves heart circulation and liver ailments (Kaul, 1967).

All the findings discussed above prove the role of honey as a valuable medication in different ailments. Most of the findings are obtained from the study done on honey of the common honeybee. Honey of the common honeybee, *Apis* spp has been investigated as a therapeutic agent by several researchers and its physiochemical properties are well – known. But honey from stingless bee that are distributed in the tropics and subtropics is less studied, even though it locally collected and used against different ailments and suppose to be superior to *Apis* honey (Torres *et al.*, 2003).

3.0 MATERIALS AND METHODS

3.1 Materials

The biochemical and several other physical properties of 'Kelulut' honey were studied. The sample was bought directly from one of Kelantan's popular honey collectors, Pok Nik Madu. According to Pok Nik, the honey was harvested from the nest of wild 'Kelulut' around the area of Chenor and Gunung Raya in Pahang, at an altitude of 2000 metres above sea level (personal communication; Pok Nik Madu, July 2004).

3.2 Methods

The 'Kelulut' honey was brought to biomedicine laboratory, School of Health Sciences, Universiti Sains Malaysia for experimental purpose. The physical and biochemical properties were investigated using the following methods:

3.2.1 Physical characteristics

The physical characteristics that were determined were the appearance, pH, specific gravity, moisture, dry matter and ash content.

3.2.1.1 Appearance of Honey

The characteristics that were determined were the color, the smell and the crystallization. Besides evaluating the color of pure honey and its dilutions at 20%, 40%, 60% and 100%, it was also determined as its optical density without dilution of honey at 420 nm with spectrophotometer. The smell was analysed by using basic sense.

The crystallization was determined by its crystallization rate at normal storage temperature.

3.2.1.2 Determination of pH

Principle

pH is an important parameter to be measured and controlled. The pH of a solution indicates how acidic or basic (alkaline) it is. The formal mathematical definition of pH is the negative logarithm of hydrogen ion concentration. In most cases, hydrogen ion activity can be approximated by the hydrogen ion concentration, and the formula becomes pH = - log10 $[H^+]$. On the pH scale, which varies from 0-14, a very acidic solution has a low pH value, a very basic solution has a high pH value, and a neutral solution has a pH of approximately 7. A pH meter with hydrogen ion (H^+) sensitive electrode is used for direct potentiometric measurement of equilibrium hydrogen ion activity.

Materials

1. Standard Buffer Solution of pH 7.0 and pH 4.0
2. pH meter (Microprocessor pH meter, HANNA Instruments)
3. 'Kelulut' honey

Procedure

1. The electrode assembly of the pH meter was dipped into the standard buffer solution of pH 7.0 taken in a clear and dry beaker.
2. The temperature correction knob was set to 28°C and the fine adjustment was made by asymmetry potentially knob to pH 7.0.
3. After wash the electrode assembly was then dipped into a solution of standard pH 4.0 and adjusted to the required pH by fine asymmetry potential knob.
4. The electrode assembly was raised, washed twice with distilled water, rinsed off with honey of the cultivars and then dipped into the honey.
5. The pH of the honey was noted.

3.2.1.3 Determination of Specific Gravity

Principle

Specific gravity is the heaviness of a substance compared to that of water, and it is expressed without units. In relationship to liquids, the term specific gravity is used to describe the weight or density of a liquid compared to an equal volume of fresh water at 4°C (4°C is essentially the temperature at which water is densest). If the liquid that are comparing will float on this water, it has a specific gravity of less than one. If it sinks into the fresh water the specific gravity is more than one. Fresh water at 4°C (39° F) has been assigned a value of one.

The specific gravity of honey was determined by means of a specific gravity bottle using the formula (Khalil, 2001)

$$\text{Specific gravity} = \frac{\text{Weight of honey in bottle}}{\text{Weight of distilled water in bottle}}$$

Materials

1. Specific gravity bottle, 10 ml.
2. Electric balance (Dragon 204, Mettler-Toledo Group).
3. Constant temperature water bath (Memmert).

Procedure

1. The specific gravity bottle was cleaned, dried and weighed.
2. The bottle was filled with distilled water which was boiled and cooled at room temperature for avoiding the formation of bubbles.
3. It was then immersed in a constant temperature water bath and heated for half and hour at 25°C.
4. The bottle was removed from the bath, wiped dry with tissue paper and allowed to stand for 15 minutes at room temperature.
5. The bottle with its content was then weighed again using electrical balance.
6. The same procedure was followed by replacing the water of the bottle with honeys and finally determined the specific gravity using the formula as given above.

3.2.1.4 Determination of Moisture Content

Principle

Moisture content was determined by evaporation method. These methods rely on measuring the mass of water in a known mass of sample. The moisture content is determined by measuring the mass of a food before and after the water is removed by evaporation.

$$\text{Percentage of Moisture} = \frac{M_{\text{Initial}} - M_{\text{Dried}}}{M_{\text{Initial}}} \times 100$$

Here, $M_{Initial}$ and M_{Dried} are the mass of the sample before and after drying, respectively. The basic principle of this technique is that water has a lower boiling point than the other major components within foods, e.g., lipids, proteins, carbohydrates and minerals. To obtain an accurate measurement of the moisture content of a food using evaporation methods it is necessary to remove all of the water molecules that were originally present in the food, without changing the mass of the food matrix. This is often extremely difficult to achieve in practice because the high temperatures or long times required to remove all of the water molecules would lead to changes in the mass of the food matrix, e.g., due to volatilization or chemical changes of some components. For this reason, the drying conditions used in evaporation methods are usually standardized in terms of temperature and time so as to obtain results that are as accurate and reproducible as possible given the practical constraints.

Materials

1. Evaporating plate.
2. Electric balance (Dragon 204, Mettler-Toledo Group).
3. Oven (Memmert).
4. Dessicator (Kartell).

Procedure

1. 1 gm of honey was weighed in an evaporating plate (which was previously cleaned and heated to about 100°C, cooled and weighted).
2. The evaporating plate with the honey was heated in an oven for about six hours at 100°C.
3. It was then cooled in a dessicator and weighed again.
4. Finally the percent of moisture content was determined by using the formula as given above.

3.2.1.5 Determination of Ash

Principle

The ash of a foodstuff is the inorganic residue remaining after the organic matter has been burnt away. Hence ash content can be determined by incinerating a known quantity of foodstuff, previously dried until constant weight is obtained. Ashing should not be done at temperatures exceeding 650^{0}C, at which temperatures inorganic salts like alkali chlorides will volatilize. Moreover, a portion of the ash will fuse and enclose some carbon, preventing them from being ignited.

The percentage ash was determined by using the following formula

$$\text{Percentage of Ash} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample}} \times 100$$

Materials

1. Porcelain crucible.
2. Muffle furnace (6000 Furnace, Barnstead/ Thermolyne).
3. Electric balance (Dragon 204, Mettler-Toledo Group).
4. Dessicator (Kartell).

Procedure

1. A porcelain crucible was dried in an oven at 105^{0}C for an hour. Then it was cooled in a dessicator and weighed soon after it had been adapted to room temperature.
2. 1-2 g of honey was weighed into the porcelain crucible.
3. The honey was heated on the hot plate until all the water was removed and ceased smoking.
4. The porcelain crucible was placed in cold muffle furnace and brought to temperature to 550^{0}C.
5. The sample was incinerated until a whitish or greyish ash was obtained.
6. The porcelain crucible was removed, cooled in a dessicator and weighed soon after it attaining room temperature.
7. The sample was replaced in muffle furnace and continued heating until weight was constant.

8. Total ash content of the food was then calculated, according to the formula given.

3.2.2 Biochemical Characteristics

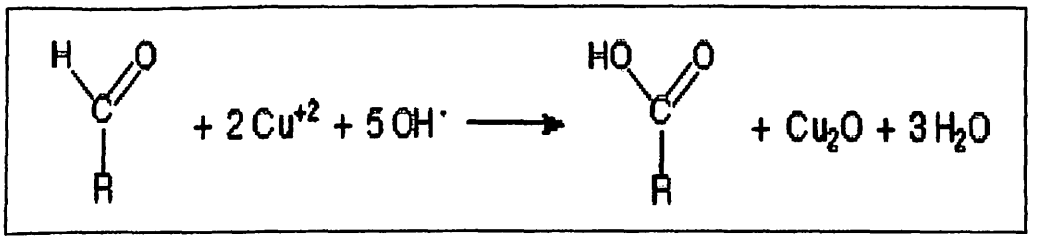
The biochemical characteristics that were determined were reducing sugar, total sugar, total protein, lipid and minerals content.

3.2.2.1 Determination of Reducing Sugar

The reducing sugar content of 'Kelulut' honey was determined by Modification of Benedict's test as a quantitative method.

Principle

Benedict's reagent is used as a simple test for reducing sugars. Benedict's reagent is a solution of copper sulfate, sodium hydroxide, and tartaric acid. Reducing sugars have either a free aldehyde functional group or a free ketone functional group as part of their molecular structure. When Benedict's reagent is heated with a reactive sugar such as glucose or maltose, reducing sugars are oxidized by the copper ion in solution to form a carboxylic acid and a brick red precipitate of copper (I) oxide.



The color of the reagent changes from blue to green to yellow to reddish-orange, depending on the amount of reactive sugar present. Orange and red indicate the highest proportion of these sugars. In the Modification of Benedict's test, the brick red precipitate of Cu (I) oxide is allowed to settle to the bottom of the tube for overnight. Then, the intensity of the unprecipitated blue Cu (II) ions is measured using a spectrophotometer at 600 nm.

Materials

1. Standard Benedict's Solution.
2. Spectrophotometer (Spectro 230, Digital Spectrophotometer, LaboMed, Inc.).
3. 'Kelulut' honey.

Procedure

Sample Preparation

1. 1 ml of 'Kelulut' honey was pipetted into the 500 ml volumetric flask and diluted to the volume by distilled water.
2. 5 ml of the diluted honey was then pipetted into the test tube and 5 ml of Benedict's solution was added and mixed well.
3. The test tube was heated for 6 minutes in a boiling water bath and cooled for overnight to allow the precipitated Cu (I) to settle to the bottom of the tube.

Standard Curve Preparation

1. A standard curve was prepared by using 0.02 M standard glucose solution to make up samples ranging in concentration from 2 - 20mM.

2. To 5 ml of each dilution, 5 ml of Benedict's solution was added and mixed well. All these solutions were treated similarly as the honey sample described above.
3. On the next day, the intensity of the unprecipitated blue Cu (II) was measured using a spectrophotometer at 600 nm.
4. A graph of absorbance of light against concentration of glucose was plotted.

Calculation

Percentage of reducing sugar was calculated using the formula below:

$$\text{Amount of sugar determined (g/ml)} \times \text{Dilution factor} \times \frac{\text{Sample volume (ml)}}{\text{Sample weight (g)}} \times 100$$

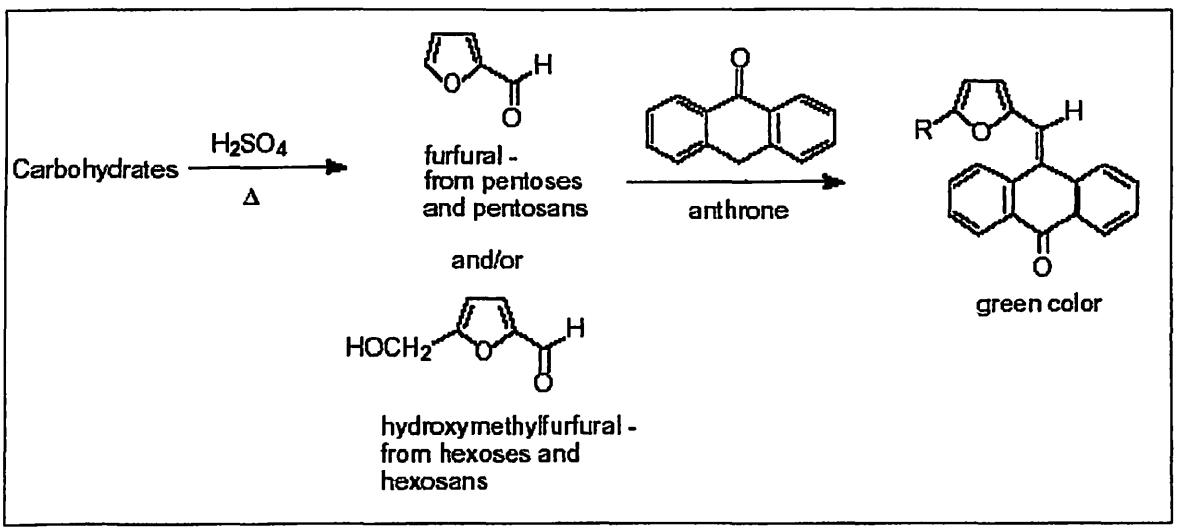
3.2.2.2 Determination of Total Sugar

Total sugar content of honeys was determined colorimetrically by the anthrone method as described in Laboratory Manual in Biochemistry (Jayaraman, 1981).

Principle

A solution of Anthrone in 95% sulphuric acid produces a characteristic blue – green color when added to solution containing carbohydrates. The sample is mixed with sulphuric acid and the anthrone reagent and then boiled until the reaction is completed. The intensity of color produced is directly proportional to the total sugar concentration in the test sample and are measured using spectrophotometer at 680 nm. This method determines both reducing and non-reducing sugars even when

these are chemically combined because of the presence of the strongly oxidizing sulfuric acid. Prior hydrolysis to convert sugars state is not needed, thus the anthrone reagent can be used for the quick determination of total sugar.



Materials

1. Anthrone reagent.

   The anthrone reagent was prepared by dissolving 2 gms of Anthrone in 1 liter of concentrated H_2SO_4.

2. Standard Glucose Solution.

   A standard glucose solution was prepared by dissolving 10 mg of glucose in 100 ml distilled water.

3. 'Kelulut' honey.

4. Spectrophotometer (Spectro 230, Digital Spectrophotometer, LaboMed, Inc.).

Procedure

Sample Preparation

1. 1 ml of 'Kelulut' honey was pipetted into the 2000 ml volumetric flask and diluted to the volume by distilled water.
2. 1 ml of diluted honey was pipetted into the test tube and 4 ml of the Anthrone reagent was added and mixed well.
3. Glass marbles were placed on the top of the tube to prevent loss of water by evaporation during the boiling.
4. The test tube were heated for 10 minutes in a boiling water bath and then cooled.
5. A reagent blank was prepared by taking 1 ml of distilled water and 4 ml of Anthrone reagent in a tube and treated similarly.

Standard Curve Preparation

1. A standard curve was prepared by using 120 M standard glucose solution to make up samples ranging in concentration from 30 M to 120 M.
2. To 1 ml of each dilution, 4 ml of Anthrone reagent was added and mixed well. All these solutions were treated similarly as the honey sample described above.
3. The absorbance of the blue green solution was measured at 680 nm in a spectrophotometer.
4. A graph of absorbance of light against concentration of total sugar was plotted.

Calculation

The percentage of total sugar present in the honeys was determined using the formula given below:

$$\text{Amount of sugar determined (g/ml)} \times \text{Dilution factor} \times \frac{\text{Sample volume (ml)}}{\text{Sample weight (g)}} \times 100$$

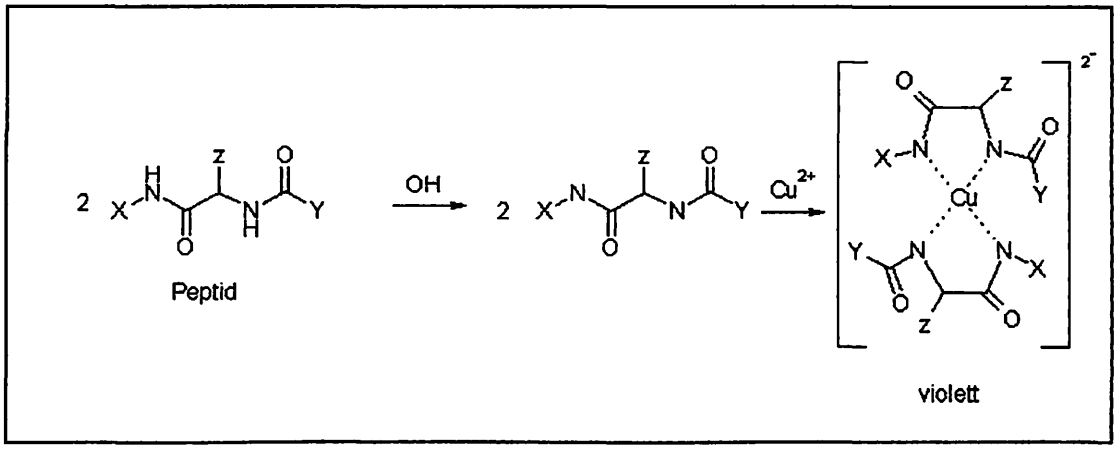
3.2.2.3 Determination of Total Protein Content

Protein content of honey was determined by Biuret test.

Principle

Proteins form a purple colored complex with cupric ions in alkaline solution. The Biuret reagent is prepared by dissolving copper sulphate, tartrate salt and often potassiun iodide in water. Alkaline copper sulphate reacts with compounds containing two or more peptide bond (-CO - NH-) to give a violet – colored complex. The depth of the color obtained is a measure of the number of peptide bonds present in the protein. The intensity of the color is measured at 540 nm.

The name of the test comes from the compound biuret ($CONH_2$-NH-$CONH_2$) which reacts in the same way and gives a positive test. Biuret is formed when the urea is heated. Two molecules of urea form one molecule of Biuret. When Cu reacts with the amide linkage in Biuret, a specific complex is formed. A similar complex is formed when Cu reacts with amide bonds in proteins.



Materials

1. Biuret reagent.
2. Standard protein solution (Bovine serum albumin).
4. Spectrophotometer (Spectro 230, Digital Spectrophotometer, LaboMed, Inc.).
3. 'Kelulut' honey.

Procedure

Sample Preparation

1. 1 ml of 'Kelulut' honey was pipetted into a beaker and was made up to 5 ml using distilled water.
2. 60 µl of diluted honey was pipetted into the test tube and 3 ml of the Biuret reagent was added and mixed well by pouring back and forth twice.
3. Then, the solution was incubated for at least 12 minutes at room temperature before taking the reading.
4. The absorbance of the violet solution was determined at 540 nm in a spectrophotometer.
5. A reagent blank was prepared by taking 60 µl of distilled water and 3 ml of Biuret reagent in a tube and treated similarly.

Standard Curve Preparation

1. A standard curve was prepared by using 120 g/l bovine serum albumin as standard protein solution to make up samples ranging in concentration 20 g/l, 40 g/l, 60 g/l, 80 g/l, 100 g/l and 120 g/l.
2. To 60 µl of each dilution, 3 ml of Biuret reagent was added and mixed well. All these solutions were treated similarly as the honey sample described above.
3. The absorbance of the blue green solution was measured at 680 nm in a spectrophotometer.
4. A graph of absorbance of light against concentration of total sugar was plotted.

Calculation

The percentage of protein present in the honeys was determined using the formula given below:

$$\text{Amount of sugar determined (g/ml)} \times \text{Dilution factor} \times \frac{\text{Sample volume (ml)}}{\text{Sample weight (g)}} \times 100$$

3.2.2.4 Determination of Lipid Content

Lipid content of honey was determined by the Bligh and Dyer method.

Principle

The Bligh and Dyer total lipid extraction is a standard method for determining total lipid in foods (AOAC, 1995). It involves homogenization of the food with chloroform and methanol. Bligh and Dyer method is suitable in measuring the lipid