PHARMACOGNOSTICAL AND PHYSICOCHEMICAL INVESTIGATION OF LOCAL COMMERCIAL HONEY

By

NG CHOI WAN

A project report submitted to Department of Biomedical Science

Faculty of Science

Universiti Tunku Abdul Rahman

In partial fulfilment of the requirements for the degree of

Bachelor of Science (Hons) Biomedical Science

April 2017

ABSTRACT

PHARMACOGNOSTICAL AND PHYSICOCHEMICAL INVESTIGATION OF LOCAL COMMERCIAL HONEY

NG CHOI WAN

The demand for honey products in the market is very high due to its medicinal values. However, honey production is always affected by environmental and climate factors. Therefore, adulterated and low quality honey can be found in the market which might not provide expected beneficial health effect or even bring harmful effect to the consumers. Hence, the main objective of this project was to investigate the pharmacognostical values and physicochemical properties of local commercial honey. Based on the result of agar well diffusion assay, Cameron Highland Wild honey exhibited great antibacterial activity against… Results obtained were compared with CODEX STAN 12-1981 standard but not all the honey samples was complied with the standards especially the moisture content, reducing sugar content, sucrose content, proline level, hydroximethylfurfural level and diastase enzyme level, which suggested due to the excessive heating of honey samples.

ACKNOWLEDGEMENT

First of all, I would like to express my sincere appreciation to my supervisor, Mr. Ng Wen Jie as well as my co-supervisor, Dr. Ee Kah Yaw for the continuous support and guidance throughout this project. I have gained a lot of experience and valuable knowledge from them and I am truly grateful for every advices given. This project also would not be so successful without their guidance.

Secondly, I would also like to express my gratitude to our lab officers, Mr. Gee Siew Meng, Mr. Tie Shin Wei as well as Mr. Saravanan a/l Sivasangaran for their patience in helping me to solve my problems during my bench work. Furthermore, I would like to express my special thanks to my bench mate, Lim Ziyun for her encouragement and supports throughout this project. We have learned a lot from each other through this project.

Besides that, I would like to thank Universiti Tunku Abdul Rahman for providing me this golden opportunity to carry out this project. Such individual project helped me to become more self-dependent and also allowed me to gain a lot of experience. Finally, I am grateful to my family who have supported me throughout my university life.

DECLARATION

I hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for other degree at UTAR or other institutions.

_____

NG CHOI WAN

APPROVAL SHEET

This project report entitled "**PHARMACOGNOSTICAL AND PHYSICOCHEMICAL INVESTIGATION OF LOCAL COMMERCIAL HONEY.**" was prepared by NG CHOI WAN and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Biomedical Science at Universiti Tunku Abdul Rahman.

Approved by:

_____

(Mr. NG WEN JIE) Date: ……...

Supervisor

Department of Biomedical Science

Faculty of Science

Universiti Tunku Abdul Rahman

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: _____

PERMISSION SHEET

It is hereby certified that **NG CHOI WAN** (ID No: **13ADB05016**) has completed this final year project entitled "PHARMACOGNOSTICAL AND PHYSICOCHEMICAL INVESTIGATION OF LOCAL COMMERCIAL HONEY." Under the supervision of Mr. Ng Wen Jie from the Department of Biomedical Science, Faculty of Science, and Dr. Ee Kah Yaw from the Department of Agriculture and Food Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project/ dissertation/ thesis* in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

_____

(NG CHOI WAN)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ΔE^*_{ab}	Total colour difference
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
AEAC	Antioxidant equivalent ascorbic acid content
$AlCl_3$	Aluminium chloride
CEQ	Catechin equivalent
CFU	Colony forming units
DHA	Dihydroxyacetone
DNSA	3,5-dinitrosalicylic acid
DPPH	2, 2-diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
Fe^{2+}-TPTZ	Ferrous tripyridyltriazine
Fe^{3+}-TPTZ	Ferric tripyridyltriazine
$FeCl_3$	Ferric chloride
GAE	Gallic acid equivalent
H_2O_2	Hydrogen peroxide
HFCS	High fructose corn syrup
HMF	Hydroxymethylfurfural
MGO	Methylglyoxal
MRSA	Methicillin-resistant *Staphylococcus aureus*
$NaNO_2$	Sodium nitrate

NaOH	Sodium hydroxide
TPTZ	2, 4, 6-tri[2-pyridyl]-s-triazine
PBS	Phosphate-buffered saline
RSA	Radical scavenging activity
VRE	Vancomycin-resistant *Enterococci*

CHAPTER 1

INTRODUCTION

According to Singh, et al. (2012), honey is a natural sweet liquid produced by honey bees via modifications of nectar and sweet deposits that collected from plants. Honey is mainly consisted of sugars as well as water with fructose and glucose as the main carbohydrate constituents. Besides that, honey contains several important substances especially the phytochemicals, vitamins and enzymes that are responsible for the antioxidant properties (Rane and Doke, 2014). Apart from that, several reports showed that natural honey exhibits broad spectrum antibacterial properties after tested on pathogenic bacteria and food spoilage bacteria. Thus, the presence of antioxidant and antibacterial effects makes honey to be classified as a medical natural product (Mandal and Mandal, 2011).

According to Elflein (2013), due to the arising awareness about the benefits of honey for human health, it leads public concerns about the quality of honey in the market. He also stated that there are several marketing factors that affecting honey quality including the low profit margin and low retail price for commercial honey blends, increasing market demands for honey supply and honey supply shortage due to global warming or bee diseases. This causes…..

CHAPTER 2

LITERATURE REVIEW

2.1 Honey

2.1.1 Introduction

Honey is a highly concentrated product of nectar and sweet deposits from different floral sources that are modified and stored by the bees in honey combs (Umarani, et al., 2015). Based on the sources, honey can be classified into blossom honey that obtained mainly from flower nectars, honeydew honey that collected from honeydew, or monofloral honey that collected predominantly from one plant type and finally the multifloral honey with several floral sources (Alvarez-Suarez, et al., 2014). During honey collection, nectar was sucked by the bee via insertion of its proboscis into flower nectar. The nectar collected will pass through esophagus of the bee into thorax and finally into the abdomen. After that, nectar will be transported into wax honeycomb cells in the bee hive. Excess water in nectar will be evaporated by the bee wings in order to maintain the ventilation of bee hive. Lastly the final product which is the honey with approximately 83% sugar and 17% water is formed. Although sucrose is the main sugar constituent in nectar, but honey consists mainly glucose and fructose due to the action of bee enzyme invertase that breaking down sucrose into simple sugars glucose and fructose (Olaitan, Adeleke and Ola, 2007).

According to Alvarez-Suarez, et al. (2014), honey is considered as a natural source of both macronutrient and micronutrient. Other than glucose and

fructose, there are more than 22 types of sugars found in honey. Besides that, honey also contains more than 180 types of other substances including amino acids, enzymes, minerals, proteins, ash, organic acids and phenol compounds. However, the components and properties of honey vary due to botanical, geographical and environmental factors (Sohaimy, Masry and Shehata, 2015).

2.1.2 Honey in Malaysia

Several places in Malaysia like Baru Pahat in Johor, Bagan Datuk in Perak, Kuala Langat and Kuala Selangor in Selangor are the major areas that are involved in honey production (Haridi, 2010). According to Ismail (2014), total honey production in year 2008 was 1228 metric tons while the import quantity in the same year was 6749 metric tons. According to his research, he proposed that local honey should be used for consumption instead of imported. This is because certain countries tend to keep high quality honey for their own population while exporting lower quality honey to other countries. However this accusation is still under debate. Due to this reason, locals should consider consuming local honey instead of imported honey for own health concerns and also to reduce the cost for honey importation.

However according to Lim and Baharun (2009), local honey production is slowly declining due to massive deforestation in Malaysia that damaging the natural habitat of bees. Besides that, they also stated that honey production is affected by rainfall since heavy rain may drain away the floral nectars, thus diminishing the amount of honey that can be collected. Rainfall also leads to high humidity in the environment which affecting the ripening of nectar and

promoting the growth of yeast that affecting the quality of honey. This causing lower amount of honey production in Malaysia as compared to other countries, thus importing large amount of honey from Australia, China and United States is still necessary in order to cope with the market demand in Malaysia (Lim and Baharun, 2009).

Due to shortage of local honey supply, market retail price for locally produced honey is usually much higher as compared to imported honey. Both honey import and export prices in Malaysia were found to be steady in trend with the overall import price fell in between RM4.62 to RM6.98 per kg and the overall export price from RM4.65 to RM9.41 per kg in the year of 2000 to 2004. China was found to be the major honey importer to Malaysia with the lowest price as compared to Australia, New Zealand and United States. This could be due to the issue where honey from China was claimed to be contaminated with certain hazardous antibiotics in 2002 (Lim and Baharun, 2009).

2.2 Antibacterial Properties of Honey

According to Mandal and Mandal (2011), antibacterial properties of honey can be clearly observed where honey remains unspoiled even after long term of storage. The medicinal properties of honey has been recently rediscovered by the medical professions due to the failure of some conventional therapeutic agents. According to their research, Manuka honey (*Leptospermum* species) was found to exhibit antibacterial activities against several pathogenic bacteria including *Staphylococcus aureus*, *Helicobacter pylori*, *Enterobacter aerogenes* and *Salmonella typhimurium*. Apart from that, their study also showed that

honey was effective against methicillin-resistant *S. aureus* (MRSA), β-haemolytic *Streptococci* and vancomycin-resistant *Enterococci* (VRE). Scientific evidences above explained the reason of using honey as an alternative treatment when conventional therapeutic methods failed to eliminate infections. However, the antibacterial properties of honey can be different up to 100-fold due to geographical, botanical and seasonal factors that affect the physiology of floral sources (Mandal and Mandal, 2011).

Since honey is a product with concentrated sugar solution, therefore its low water activity is able to inhibit the growth of microorganisms including bacteria, yeasts and molds. High osmotic pressure of honey causes water withdrawal from wounds when it is applied topically, thus inhibiting bacterial growth and preventing further infections (Naama, 2009).

Antibacterial action of honey is mainly due to the action of hydrogen peroxide, which is a product of glucose oxidase enzymatic reaction. However, non-peroxide antibacterial properties can be found in honey as well (Irish, Blair and Carter, 2011). Such honey is known as non-peroxide honey which is able to express its antibacterial activity even without the presence of hydrogen peroxide which is advantageous for human as the antibacterial activity will not be neutralized by the catalase activity in body. One of the non-peroxide components that is responsible for the antibacterial activity in honey is methylglyoxal (MGO). Other than hydrogen peroxide and MGO, antimicrobial peptide bee defensin 1 is also involved in the antibacterial mechanism of honey (Mandal and Mandal, 2011).

According to Kwakman, et al. (2011), MGO is present exclusively high in Manuka honey but certain amount of MGO can also formed from sugars due to heat treatment or prolonged storage. Dihydroxyacetone (DHA) is a compound found exclusively in the nectar of Manuka tree whereby it can be converted into MGO through natural chemical process. However this conversion often requires three to four years in suitable temperature which is 22 °C (Wearmouth, 2015). Bee defensin 1 is a type of peptide composed of 51 amino acids with molecular weight of 5.5 kDa. This peptide has prominent antibacterial effect against Gram-positive bacteria as compared to Gram-negative bacteria. Bee defensin 1 can be found in most of the honey including Manuka honey with different quantities (Valachova, Bucekova and Majtan, 2016).

2.3 Antioxidant Properties of Honey

The presence of antioxidants in food product is known as the "fountain of youth" due to the effects in reducing the risk of many diseases such as stroke, cancer, cataract, heart problem and in slowing the process of ageing as well (Khalil, Sulaiman and Boukraa, 2010). As the name proposed, antioxidants are the compounds that inhibiting oxidation process. Although oxygen is required for the survival of organisms, but excessive amount of oxygen may form free oxygen radicals that are toxic to the cells. Therefore, antioxidants are needed to serve as radical scavengers, which convert reactive radicals to less reactive form and prevent reaction with body cells to avoid cell damage (Kumar, 2014). According to Khalil, Sulaiman and Boukraa (2010), phytochemicals such as polyphenols are the main contributors to the antioxidant effects in honey.

Polyphenols are consist of flavonoids and phenolic acid, which attributed to the antioxidant properties of honey together with other constituents such as organic acids, amino acids, proteins, Maillard reaction products, carotenoid like compounds, enzyme glucose oxidase and catalase. Amount of phenolic compounds in honey is usually different according on the botanical and climate conditions where the plant was located (Khalil, Sulaiman and Boukraa, 2010). However, bioavailability of polyphenols in human body can be affected by several factors including the microbiota metabolism, ability of gut absorption, plasma kinetics, excretion from urine and many others (Moussa, Saad and Noureddine, 2012).

The lacking of standardized method for the evaluation of antioxidant activity is the main reason why several antioxidant assays are needed to examine the antioxidant capacity of honey (Khalil, Sulaiman and Boukraa, 2010). There are few antioxidant assays that commonly adopted to assess the antioxidant capacity of honey including including ABTS assay, DPPH assay, antioxidant equivalent ascorbic acid content (AEAC) assay and ferric reducing antioxidant power (FRAP) assay (Khalil, Sulaiman and Boukraa, 2010). ABTS assay involves 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) which is the ABTS radicals that generated through the reaction between strong oxidizing agents such as potassium permanganate or potassium persulfate with an ABTS salt. The radicals are formed in blue-green colour therefore reduction of radicals by antioxidants that resulting in decrease of colour intensity can be measured through spectrophotometric method (Shalaby and Shanab, 2012). Apart from that, DPPH assay is performed by using 2,

2-diphenyl-1-picrylhydrazyl (DPPH) radicals to evaluate the antioxidant capacity of honey samples. DPPH is a stable radical with higher absorption at wavelength of 515 nm. When the radicals being scavenged, the absorption ability decreased. Such changes can be measured to determine the radical scavenging activity of antioxidant tested while antioxidant equivalent ascorbic acid content (AEAC) assay is another antioxidant assay with the similar principle as DPPH assay but the antioxidant potential is compared with ascorbic acid content specifically (Shalaby and Shanab, 2012). On the other hand, ferric reducing antioxidant power (FRAP) assay is a different antioxidant assay that measuring the ability of an antioxidant to react and reduce the ferric ion from ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex into ferrous ion, forming blue coloured complex of ferrous tripyridyltriazine (Fe^{2+}-TPTZ) that can be quantified using spectrophotometer (Nishaa, et al., 2012). FRAP assay is suitable for the measurement of total antioxidants present in most of the natural products since all antioxidants can react in FRAP assay except for the compounds with SH containing group such as thiol (Shalaby and Shanab, 2013).

2.4 Physicochemical and Enzymatic Properties of Honey

2.4.1 Introduction

Majority of the honey is mainly composed of glucose and fructose along with water as the second major component. However, there are other minor constituents with different quantity that present in honey which can be employed to categorize honey into different quality level (Shahnawaz, et al., 2013). On the other hand, artificial honey or adulterated honey can be produced

from any carbohydrate source that has relative similar range of glucose and fructose composition with natural honey. Normally such artificial honey will have similar taste or appearance like natural honey product which making the artificial product indistinguishable from the real natural product. However, natural honey is different from artificial honey due to the presence of the minor constituents. Normally artificial honey is lacking of certain types of constituents that affect the medicinal value of honey. Therefore, the physicochemical characterization of honey is important to differentiate between artificial honey with authentic natural honey. Besides, honey characterization is also useful to determine the quality of honey as well (James, et al., 2009).

According to Moniruzzaman, et al. (2013a), there are a few parameters that can reflect the physicochemical properties of honey including electrical conductivity, reducing and non-reducing sugars content, diastase activity, hydroxymethylfurfural (HMF) content, moisture content, ash content and free acidity.

2.4.2 Colour Intensity and Characteristic

According to National Honey Board (2017), the colour of honey is one of the features that changes variably due to the differences in botanical origin, period and condition of storage. Colour of honey ranges from pale yellow to amber, darkish red amber and to nearly dark brown. Normally honey with lighter colour comes with milder taste while dark honey has a stronger taste (National Honey Board, 2017). Colour of honey can be characterized by

spectrophotometer whereby several parameters can be revealed including components L*, a* and b*. L* represents the lightness of honey, a* represents redness while b* indicates yellowness. However, both a* and b* values could be affected by honey crystallization. The total color difference (ΔE*) can be obtained by calculation from these parameters (Zapotoczny, Kawalko and Bakier, 2010).

On the other hand, colour intensity of honey indicates the level of pigments present such as carotenoids and flavonoids which are the antioxidants that responsible for colour formation. Therefore, a strong correlation between colour intensity with the amount of antioxidants present in honey can be found (Moniruzzaman, et al., 2013a). Carotenoids are the most predominant pigments found in honey that mainly responsible for the colour formation in light colored honey (Alqarni, Owayss and Mahmoud, 2016).

2.4.3 Acidity

Honey is a natural product with acidic properties. The pH of honey usually falls between ranges of 3.2 to 4.5, which is acidic enough to inhibit the growth of many common pathogens including *Escherichia coli*, *Salmonella, Pseudomonas aeruginosa* and others. Hence, acidity is one of the main antibacterial factors in undiluted honey (Olaitan, Adeleke and Ola, 2007). Microbial growth is inhibited at low pH due to the effect of hydrogen ions concentration gradient. When concentration gradient of hydrogen ions across the plasma membrane is disrupted, it affects the proton motive force that responsible for ATP production in cells and therefore normal cellular

respiration process in bacteria is inhibited (Blamire, 2000). Low pH of honey is due to the action of glucose oxidase that converts glucose into hydrogen peroxide and gluconic acid (Kwakman, et al., 2010). Other than inhibiting bacterial growth, acidic nature of honey causes macrophage stimulation, enhancing fibroblast and increase oxygenation in wounds. Growth factor TGF-β which is important for wound recovery was also found to be more active physiologically in an acidic condition (Alvarez-Suarez, et al., 2014).

2.4.4 Viscosity

According to James, et al. (2009), viscosity can be described as the thickness or internal friction of fluid. Honey is considered as a thick liquid with low level of fluidity. Normally, honey with high quality is thicker and more viscous as compared to lower quality honey. Viscosity of honey is mainly related to its sugar composition, water together with the colloid content. Colloidal content contributes to the viscosity while higher water content and fructose level reduce the viscosity. Therefore the viscosity of each different honey is different due to the composition (James, et al., 2009). Besides, a correlation was found between honey viscosity with temperature whereby honey was more viscous in lower temperature and became more watery when temperature increased (Gomez-Diaz, Navaza and Quintans-Riveiro, 2009).

2.4.5 Electrical Conductivity and Total Dissolved Solids

Electrical conductivity is the main parameter for the authentication of unifloral honey. Higher conductivity value is due to the amount of ash and acid present in the honey (Moniruzzaman, et al., 2013b). Electrical conductivity can be used

to differentiate honey with different botanical origins as the mineral content is different based on the soil where the plant was located. This parameter is becoming one of the international standard parameters for honey to replace the measurement of ash content (Zerrouk, et al., 2011). Based on the standard from Codex Alimentarius Commission (2001), electrical conductivity for most of the honey should fall below 0.8 mS/cm but honeydew and chestnut honey could be higher than 0.8 mS/cm. Total dissolved solid is another parameter measuring both organic and inorganic compounds content in honey regardless in molecular, ionized or micro-granular suspended form. Since the presence of trace elements in honey depends on its botanical origin, therefore the measurement of total dissolved solid is also useful in differentiating honey from different sources (Sulieman, Abdelhmied and Salih, 2013).

2.4.6 Moisture Content

Water is the second major content of honey other than sugar. Water content in honey is depending on several factors including the weather, hive humidity during honey production, nectar, and the treatment given to honey during extraction process as well as the honey storage condition (Olaitan, Adeleke and Ola, 2007). The moisture content of honey should not be more than 20% according to standard from Codex Alimentarius Commission (2001). High moisture content can change the quality of honey in many aspects including flavor, preservation, crystallization, specific weight, viscosity and also responsible for the growth of fermenting microorganisms. Low moisture content in honey limits the water availability for microbial consumption. This causes dehydration in the microbes and inhibiting enzyme activity (Stark and

Firestone, 1995). The growth of microorganisms is found to be more prominent in honey with higher moisture content that leads to fermentation (Olaitan, Adeleke and Ola, 2007). Positive correlation was found between moisture content with acidity because higher water level promotes greater glucose oxidase activity that producing higher level of gluconic acid (Ananias, Melo and Moura, 2013).

2.4.7 Water Activity

Water activity is the major factor that responsible for food stability as it is referring to the unbound water molecules or water that available to support the growth of microorganisms. In contrast to water activity, moisture content is referring to the total amount of moisture in honey, including both bound and unbound water (Nielson, Bilde and Frosch, 2012). Due to the high content of sugar present in honey, the strong interactions between sugar molecules causing low amount of water available for microorganisms (Olaitan, Adeleke and Ola, 2007). Most of the bacteria is inhibited when water activity drops below 0.75 but it is not sufficient to inhibit the growth of yeasts and molds. The growth of all microorganisms only can be inhibited when water activity is below 0.6 (Court, 2006).

The water activity of honey mainly depends on the concentrations of glucose and fructose. However, glucose has the ability to crystallize into a form of glucose monohydrate when the ratio of glucose to water increases to certain extent that lowers the concentration of glucose in honey, leads to higher water activity. However, when glucose crystals are re-dissolved upon warming, the

water activity decreases again. Hence, honey with crystallization tends to have higher chance of spoilage due to high water activity that supports the growth of microorganisms. Apart from that, factors such as viscosity, storage temperature and the presence of foreign particles also can affect the crystallization of honey (Shafiq, et al., 2014).

2.4.8 Sugar Content

Honey is a viscous and highly concentrated solution with approximately 80-85% of carbohydrates with glucose and fructose which are the main sugar constituents. Usually the sugar content in honey depends on several factors: sugar content in nectar, enzymes present in the bees and the nectar. Honey with good quality should have higher fructose content than glucose to prevent crystallization (Buba, Gidado and Shugaba, 2013). Honey with low sugar content but high water content can spoil easily due to the growth and fermentation of yeast. High sugar content in honey leads to high osmolarity, which is able to inhibit the growth of microorganisms such as bacteria and fungi. Increasing surrounding osmolarity causes decrease in external osmotic pressure surrounding the cell, leads to cellular dehydration due to efflux of water (Wood, 2015). Most of the bacteria are unable to survive in high osmolarity condition, however bacteria *Staphyloccocus aureus* was found to have a higher osmolarity tolerance as compared to other bacteria (Olaitan, Adeleke and Ola, 2007). Since the survival rate of bacteria is higher when honey is diluted, therefore when honey is applied as wound dressing, the dressing has to be changed frequently due to the fluid outflow from wounds caused by hyperosmolarity effect of honey (Molan, 2012).

2.4.9 Reducing Sugars and Sucrose

Sugar composition is another parameter that can be adopted to differentiate honey according to their botanical origin since its composition is not greatly affected by the processing or storage condition (Nayik, Dar and Nanda, 2015). Reducing sugars glucose and fructose account for approximately 31.3% and 38.2% of honey composition respectively. Apart from that, low level of disaccharides such as sucrose, maltose and isomaltose contribute to the remaining percentage of sugar content in honey (Olaitan, Adeleke and Ola, 2007). Other than botanical origin, the level of sucrose also varies differently according to the degree of honey maturity (Asadi-Dizaji, et al., 2014). In a fully ripened honey, sucrose is broken down into glucose and fructose by bee enzyme invertase. Therefore high level of sucrose in honey indicates insufficient nectar processing by the enzyme (Olaitan, Adeleke and Ola, 2007). Based on Codex Alimentarius Commission (2001), the reducing sugar content of honey should be more than 60% and the sucrose content should be lower than 5%.

2.4.10 Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is another main factor contributes to the antibacterial properties in diluted honey. The presence of H_2O_2 in honey is due to the activity of enzyme glucose oxidase that is synthesized by the bees. Glucose oxidase is an enzyme that responsible for the conversion of glucose to form gluconic acid and H_2O_2 (Mandal and Mandal, 2011). According to Linley, et al. (2012), H_2O_2 functions as an antibacterial agent with the radical formation mechanism. Highly reactive hydroxyl radicals are produced through

the reaction between superoxide radicals with hydrogen peroxide as shown below:

$$O_2\bullet^- + H_2O_2 \rightarrow O_2 + OH^- + OH\bullet$$

Similar to other oxygenated species, hydroxyl radicals formed from the reaction are able to act as oxidizing agents to react with lipids, proteins and nucleic acids, causing cell damage.

The enzyme glucose oxidase has an optimum pH of 6.1 and shows higher enzymatic activity in between pH 5.5 to pH 8. The pH of undiluted honey is normally too low that can inhibit the activity of glucose oxidase, preventing production of H_2O_2. Therefore, enzyme glucose oxidase is more active in diluted honey. However, the dilution has to be performed accurately as the production of hydrogen peroxide can be affected if there is insufficient glucose due to excessive dilution. Besides that, the presence of catalase can reduce the level of hydrogen peroxide by degrading it into water and oxygen. Plus, the presence of ascorbic acid can lower the level of hydrogen peroxide through oxidation process as well (Mahmoud and Owayss, 2006).

2.4.11 Proline

There are several types of amino acids can be found in honey including proline, phynylalanine, tyrosine, lysine and arginine with proline which is the most predominant type (Hermosin, Chicon and Cabezudo, 2003). There are approximately 50% to 85% of proline present in respect to other amino acids. The amount of proline in honey is affected by bee species since the majority of

proline present is originated from the secretion of bees during nectar conversion into honey (Geronimo and Fritz, 2001). It was suggested that proline level in pure honey without adulteration should be above 180 mg/kg while proline level below 180 mg/kg suggest possibility of adulterated honey (Codex Alimentarius Commission, 2001). Honey adulterated with high fructose corn syrup (HFCS) was more diluted and therefore having lower level of proline (Geronimo and Fritz, 2001).

2.4.12 Hydroxymethyfurfural

Hydroxymethyfurfural (HMF) is a constituent that normally absent in fresh honey and therefore the level of HMF in honey is one of the parameters to determine the quality of honey. The presence of HMF increases due to prolonged storage or heat processing. This compound is formed from simple sugars especially fructose from a process known as acid-catalyzed dehydration. Three water molecules loss from fructose by the action of acid. As for HMF formation from glucose, it requires isomerization process to form fructose from glucose prior to the dehydration process. Since honey is rich with both glucose and fructose, together with acidic nature of honey, HMF can be formed easily (Kesic, et al., 2014). Heat treatment on honey is essential to reduce the viscosity of honey for easier handling and also to prevent crystallization and fermentation in order to maintain the quality of honey. Therefore higher level of HMF in honey indicates longer processing or storage duration (Zappala, et al., 2005). Based on the standards provided by Codex Alimentarius Commission (2001), HMF level after honey processing should not be exceeding 80 mg/kg.

2.4.13 Diastase Enzyme

There are a few types of enzyme present in honey that responsible for carbohydrate metabolism including diastase, invertase, glucosidase, glucose oxidase and catalase (Rossano, et al., 2012). Diastase enzyme is an enzyme secreted from bees and this enzyme is responsible for hydrolyzing starch that present in nectar into maltose and dextrins (Balasubramanyam, 2014). Diastase level is one of the parameters used to determine the freshness and heat damage of honey. According to Codex Alimentarius Commission (2001), the diastase number of honey after processing should not be lower than 8 Schade value/g. For honey adulterated with high fructose corn syrup (HFCS) containing hydrolyzed starch and inverted sucrose that can lead to reduce in diastase number due to honey dilution by HFCS. However such adulteration can be masked via addition of foreign enzyme such as bakery mould amylases (Voldrich, et al., 2009).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Honey Samples

Seven local commercial honey samples (Figure 3.1) were purchased from random retailers and stored at room temperature in dark condition to maintain the quality of honey. Honey was stored in room temperature to reduce the progress of honey crystallization while storage in dark condition could prevent excessive exposure to the sunlight that may alter the quality of honey (National Honey Board, 2017). The details of the seven honey samples are summarized as shown in Table 3.1….

3.3 Statistical Analysis

All assays was performed in triplicate and the results were expressed in mean ± standard deviation. The results were analyzed using independent sample t-test in IBM Statistical packages for Social Science (SPSS) version 20.0 for the comparison between antioxidant and physicochemical properties of tested honey samples. Significant difference between means was considered at 95% ($p < 0.05$) confidence level.

CHAPTER 4

RESULTS

4.1 Antibacterial Properties

4.2 Antioxidant Properties

4.3 Physicochemical and Enzymatic Properties

CHAPTER 5

DISCUSSION

5.1 Antibacterial Properties

5.2 Antioxidant Properties

5.3 Physicochemical and Enzymatic Properties

CHAPTER 6

CONCLUSION

In a nutshell…

REFERENCES

Abramovic, H., Jamnik, M., Burkan, L. and Kac, M., 2007. Water activity and water content in Slovenian honeys. *Science Direct*, 19(11), pp. 1086-1090.

Adenekan, M.O., Amusa, N.A., Lawal, A.O. and Okpeze, V.E., 2010. Physico-chemical and microbiological properties of honey samples obtained from Ibadan. *Journal of Microbiology and Antimicrobials*, 2(8), pp. 100-104.

Ahmed, M., Aissat, S. and Djebli, A., 2014. Effect of heat treatment on antimycotic activity of Sahara honey. *Journal of Coastal Life Medicine*, 2(11), pp. 876-881.

Ahmed, S. and Othman, N.H., 2013. Review of the medicinal effects of Tualang honey and a comparison with Manuka honey. *The Malaysian Journal of Medical Sciences*, 20(3), pp. 6-13.

Ahmed, M., Shafiq, M.I., Khaleeq, A., Huma, R., Qadir, M.A., Khalid, A., Ali, A. and Samad, A., 2016. Physiochemical, biochemical, minerals content analysis, and antioxidant potential of national and international honeys in Pakistan. *Journal of Chemistry*, 6(10), pp. 1-10.

Aljadi A.M. and Kamaruddin M.Y., 2004. Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chemistry*, 85, pp. 513–518.

Alqarni, A.S., Owayss, A.A. and Mahmoud, A.A., 2016. Physicochemical characteristics, total phenols and pigments of national and international honeys in Saudi Arabia. *Arabian Journal of Chemistry*, 9(1), pp. 119-120.

Alvarez-Suarez, J.M., Gasparrini, M., Forbes-Hernández, T.Y., Mazzoni, L. and Giampieri, F., 2014. The composition and biological activity of honey: a focus on manuka honey. *Foods Journal*, 3(3), pp. 420-422.

Alzahrani, H.A., Boukraa, L., Bellik, Y., Abdellah, F., Bakhotmah, B.A., Kolayli, S. and Sahin, H., 2012. Evaluation of the antioxidant activity of three varieties of honey from different botanical and geographical origins. *Global Journal of Health Science*, 4(6), pp. 191-196.

Ananias, K.R., Melo, A.A.M. and Moura, C.J., 2013. Analysis of moisture content, acidity and contamination by yeast and molds in *Apis mellifera* L. honey from central Brazil. *Brazilian Journal of Microbiology*, 44(3), pp. 681-682.

Asadi-Dizaji, A., Moeni-Alishah, F., Yamini, Y., Ebrahimnezhad, Y., Yari, A.A. and Rouhnavaz, S., 2014. Physico-chemical properties in honey from different zonal of East Azerbaijan. *Biological Forum-An International Journal*, 6(2), pp. 205-206.

Asaduzzaman, M., Rahman, M.S., Munira, S., Rahman, M.M., Islam, M., Hasan, M., Siddique, M.A.H., Biswas, S., Khan, M.H., Islan, M.A. and Rahman, M., 2015. Analysis of biochemical composition of honey and its anti-oxidant, phytochemical and anti-bacterial properties. *Journal of Biomedical and Pharmaceutical Research*, 4(4), pp. 69-81.

Assia, A. and Ali, L., 2015. Enzymes activities, hydroxymethylfurfural content and pollen spectrum of some Algerian honey. *African Journal of Agricultural Research*, 10(7), pp. 613-622.

Balasubramanyam, M.V., 2014. Role of different enzymes in nectar to honey transformations in indigenous rockbee, *Apis Dorsata*. *Journal of Chemical, Biological and Physical Sciences*, 4(1), pp. 364-365.

Balasubramanyam, M.V., 2016. Detection of laevulose, dextrose and sucrose levels in ripening of honey in dwarf bee, *Apis Florea*. *Journal of Chemical, Biological and Physical Sciences*, 7(1), pp. 49-54.

Benzie, I.F.F. and Strain, J.J., 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology*, 1999(299), pp. 15-27.

Beretta, G., Granata, P., Ferrero, M., Orioli, M., & Facino, R. M. (2005). Standardization of antioxidant properties of honey by a combination of spectrophotometric or fluorimetric assays and chemometrics. *Analytica Chimica Acta*, 533(6), 185-191.

Bizerra, F.C., Silva, P.I.D. and Hayashi, M.A.F., 2012. Exploring the antibacterial properties of honey and its potential. *Frontiers in Microbiology*, 3(398), pp. 1-2.

Blamire, J., 2000. *Effect of pH on growth rate.* [Online] Available at: http://www.brooklyn.cuny.edu/bc/ahp/CellBio/Growth/MGpH.html [Accessed 13 March 2017].

Boateng, J. and Diunase, K.N., 2015. Comparing the antibacterial and functional properties of Cameroonian and Manuka honeys for potential wound healing - have we come full cycle in dealing with antibiotic resistance? *Molecules*, 20(9), pp. 16068-16084.

Boussaid, A., Chouaibi, M., Rezig, L., Hellal, R., Donsi, F., Ferrari, G. and Hamdi, S., 2014. Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia. *Arabian Journal of Chemistry*, 2(10), pp. 1-10.

Buba, F., Gidado, A. and Shugaba, A., 2013. Analysis of biochemical composition of honey samples from North-East Nigeria. *Biochemistry & Analytical Biochemistry*, 2(3), pp. 1-7.

Bucekova, M., Valachova, I., Kohutova, L., Prochazka, E., Klaudiny, J. and Majtan, J., 2014. Honeybee glucose oxidase--its expression in honeybee workers and comparative analyses of its content and H_2O_2-mediated antibacterial activity in natural honeys. *The Science of Nature*, 101(8), pp. 661-700.

Burns, D., 2007. *Lesson twelve: the moisture level of honey*. [Online] Available at: http://basicbeekeeping.blogspot.my/2007/10/lesson-twelve-moisture-level-of-h

oney.html [Accessed 5 April 2017].

Chua, L.S., Rahaman, N.L., Adnan, N.A. and Tan, T.T., 2013. Antioxidant activity of three honey samples in relation with their biochemical components. *Journal of Analytical Methods in Chemistry, 2013(2013)*, pp. 1-8.

Codex Allimentarius Commission, 2001. *Codex STAN 12 – 1981*. [Online.] Available at: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCODEX%2BSTAN%2B12-1981%252Fcxs_012e.pdf [Accessed 10 March 2017].

Court, N., 2006. *Fundamentals of water activity.* [Online] Available at: http://nfscfaculty.tamu.edu/talcott/courses/FSTC605/Papers%20Reviewed/Fundamentals%20of%20Aw-Decagon.pdf [Accessed 30 March 2017].

Cozmuta, A.M., Cozmuta, L., Varga, C., Marian, M. and Peter, A., 2011. Effect of thermal processing on quality of polyfloral honey. *Romanian Journal of Food Science*, 1(1), pp. 45-52.

Czipa, N., Zoltan, G. and Borbely, M., 2012. Proline content of different honey types. *Acta Alimentaria*, 41(1), pp. 26.32.

Elflein, L., 2013. *Current issues and trends of honey quality in the global honey market*. [Online] Available at: https://www.apimondia.com/congresses/2013/Technology-Quality/Plenary-Session/Current%20Issues%20and%20Trends%20Of%20Honey%20Quality%20in%20the%20Global%20Honey%20Market%20-%20Lutz%20Elflein.pdf [Accessed 6 January 2017].

Eteraf-Oskouei, T. and Najafi, M., 2012. Traditional and modern uses of natural honey in human diseases: a review. *Iranian Journal of Basic Medical Sciences*, 16(6), pp. 736-737.

Geronimo, J.D. and Fritz, R., 2001. Proline in Argentine honeys. *In:*

Conference Planners, *37th International Apicultural Congress*. South Africa, 28 October-1 November 2001. South Africa: Document Transformation Technologies.

Gomez-Diaz, D., Navaza, J.M. and Quintans-Riveiro, L.C., 2009. Effect of temperature on the viscosity of honey. *International Journal of Food Properties*, 12(2), pp. 396-404.

Haridi, A., 2010. *Beekeeping in Malaysia*. [Online] Available at: http://azzedine-haridi-bees.blogspot.my/ [Accessed 6 January 2017].

Harsh, A., Sharma, Y.K., Joshi, U., Rampuria, S., Singh, G., Kumar, S. and Sharma, R., 2016. Effect of short-term heat stress on total sugars, proline and some antioxidant enzymes in moth bean (*Vigna aconitifolia*). *Annals of Agricultural Sciences*, 61(1), pp. 57-64.

Hermosin, I., Chicon, R.M. and Cabezudo, M.D., 2003. Free amino acid composition and botanical origin of honey. *Food Chemistry*, 83(2), pp. 263.

International Honey Commission, 2009. Harmonised method of the international honey commission. [Online] Available at: http://www.ihc-platform.net/ihcmethods2009.pdf [Accessed 1 October 2016].

Irish, J., Blair, S. and Carter, D.A., 2011. The antibacterial activity of honey derived from Australian flora. *Plos One*, 6(3), pp. 1-2.

Ismail, M.M., 2014. *Competitiveness of beekeeping industry in Malaysia*. [Online] Available at: http://psasir.upm.edu.my/41608/2/COMPETITIVENESS.pdf [Accessed 13 March 2017].

James, O.O., Mesubi, M.A., Usman, L.A., Yeye, S.O., Ajanaku, K.O., Ogunniran, K.O., Ajani, O.O. and Siyanbola, T.O., 2009. Physical characterisation of some honey samples from North-Central Nigeria. *International Journal of Physical Sciences*, 4(9), pp. 464-465.

Kek, S.P., Chin, N.L., Yusof, Y.A., Tan, S.W. and Chua, L.S., 2014. Total phenolic contents and colour intensity of Malaysian honeys from the *Apis* spp. and *Trigona* spp. bees. *Agriculture and Agricultural Science Procedia*, 2(2014), pp. 150-155.

Keppy, N.K. and Allen, M.W., 2009. *The Determination of HMF in Honey with an Evolution Array UV-Visible Spectrophotometer*. Madison, WI, USA: Thermo Fisher Scientific Instrument LLC.

Kesic, A., Crnkic, A., Hodzic, Z., Ibrisimovic, N. and Sestan, A., 2014. Effects of botanical origin and ageing on HMF content in bee honey. *Journal of Scientific Research & Reports*, 3(8), pp. 1057-1058.

Khalil, M.I., Alam, N., Moniruzzaman, M., Sulaiman, S. and Gan S., 2011. Phenolic acid composition and antioxidant properties of Malaysian honeys. *Journal of Food Science*, 76(6), pp. 921-928.

Khalil, M.I., Sulaiman, S.A. and Boukraa, L., 2010. Antioxidant properties of honey and its role in preventing health disorder. *The Open Nutraceuticals Journal*, 3(6), pp.6-16.

Konica Minolta, 2017. *Identifying color differences using L*a*b* or L*C*H* coordinates.* [Online] Available at: http://sensing.konicaminolta.us/2014/04/identifying-color-differences-using-l-a-b-or-l-c-h-coordinates/ [Accessed 13 March 2017].

Kumar, S., 2014. The importance of antioxidant and their role in pharmaceutical science. *Asian Journal of Research in Chemistry and Pharmaceutical Sciences*, 1(1), pp. 27-44.

Kwakman, P.H.S., Velde, A.J., Boer, L., Christina, M.J.E., Vandenbroucke, G. and Zaat, S.A.J., 2011. Two major medicinal honeys have different mechanisms of bactericidal activity. *Plos One*, 6(3), pp. 1-7.

Kwakman, P.H.S., Velde, A.J., Boer, L., Speijer, D., Christina, M.J.E. and Zaat, S.A.J., 2010. How honey kills bacteria. *The FASEB Journal*, 24(7), pp. 2576-2582.

Lichtenberg, K.B., 2014. Evidence for correlation between invertase activity and sucrose content during the ripening process of honey. *Journal of Apicultural Research*, 53(3), pp. 364-373.
Lim, Y.W. and Baharun, R., 2009. Agriculture strategies in Malaysia: planning implementation. *Contemporary Issues in Marketing*, 88(3), pp. 39-43.

Linley, E., Denyer, S.P., Mcdonnell, G., Simons, C. and Maillard, J.Y., 2012. Use of hydrogen peroxide as a biocide: new consideration of its mechanisms of biocidal action. *Journal of Antimicrobial Chemotherapy Advance*, 67(7), pp. 1-8.

Mahmoud, A.A. and Owayss, A.A., 2006. A modified method to determine hydrogen peroxide activity as a criterion for bee honey quality. *Annals of Agricultural Sciences*, 44(4), pp. 1629-1639.

Mandal, M.D. and Mandal S., 2011. Honey: its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine*, 1(2), pp. 154-160.

Meda, A., Lamien, C., Romito, M., Millogo, J. and Nacoulma, O., 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry*, 91(3), pp. 571-577.

Megazyme, 2007. *Diastase activity (α-amylase) in honey*: assay procedure.

Meinen, N., Camilleri, L. and Attard, E., 2014. The antioxidant capacity of maltese honey. *Journal of Apiculture Sciences*, 58(1), pp. 51-60.

Mohamed, M., Sirajudeen, K.N.S., Swamy, M., Yaacob, N.S. and Sulaiman, S.A., 2010. Studies on the antioxidant properties of tualang honey of Malaysia. *African Journal of Traditional, Complementary and Alternative Medicines*, 7(1), pp. 59-63.

Mohapatra, D., Thakur, V. and Brar, S., 2011. Antibacterial efficacy of raw and processed honey. *Biotechnology Research International*, 2011(2011), pp.1-6.

Molan, P., 2012. *The antibacterial activity of honey and its role in treating diseases*. [Online] Available at: http://www.aniwell-uk.com/files/4614/3519/6994/Pdf_6_ANTIBACTERIAL_ACTIVITY_-libre.pdf [Accessed 13 January 2017].

Moniruzzaman, M., Khalil, M.I., Sulaiman, S.A. and Gan, S.H., 2013a. Physicochemical and antioxidant properties of Malaysian honeys produced by *Apis cerana*, *Apis dorsata* and *Apis mellifera*. *BMC Complementary and Alternative Medicine*, 13(43), pp. 1-12.

Moniruzzaman, M., Khalil, M.I., Sulaiman, S.A. and Gan, S.H., 2013b. Evaluation of physicochemical and antioxidant properties of sourwood and other Malaysian honeys: a comparison with manuka honey. *Chemistry Central Journal*, 7(138), pp. 5-6.

Moussa, A., Saad, A. and Noureddine, D., 2012. How honey acts as an antioxidant? *Medicinal & Aromatic Plants*, 1(5), pp. 1-2.

Naama, R.T., 2009. Evaluation of in-vitro inhibitory effect of honey on some microbial isolate. *Journal of Bacteriology Research*, 1(6), pp. 64-65.

National Honey Board, 2017. *Honey colour and flavour*. [Online] Available at: https://www.honey.com/newsroom/press-kits/honey-color-and-flavor [Accessed 18 January 2017].

National Honey Board, 2017. *Honey usage and storage tips.* [Online] Available at: https://www.honey.com/honey-at-home/tools-tips-and-resources/honey-usage-and-storage-tips/ [Accessed 13 March 2017].

Nayik, G.A., Dar, B.N. and Nanda, V., 2015. Physico-chemical, rheological and sugar profile of different unifloral honeys from Kashmir valley of India. *Arabian Journal of Chemistry*, 2(10), pp. 1-12.

Ng, W.J., Ken, K.W., Gunasagaran, H., Chandramogan, V. and Lee, Y.Y., 2014. In-vitro screening of Malaysian honey from different floral sources for antibacterial activity on human pathogenic bacteria. *African Journal of Traditional, Complementary and Alternative Medicine*, 11(2), pp. 315-318.

Nielson, O.F., Bilde, M. and Frosch, M., 2012. Water activity. *Journal of Spectroscopy*, 27(5), pp. 565-569.

Nishaa, S., Vishnupriya, M., Sasikumar, J.M., Christabel, H.P. and Gopalakrishnan, V.K., 2012. Antioxidant activity of ethanolic extract of *Maranta arundinacea L.Tuberous Rhizomes*. *Asian Journal of Pharmaceutical and Clinical Research*, 5(4), pp. 85-88.

Olaitan, P.B., Adeleke, O.E. and Ola, L.O., 2007. Honey: a reservoir for microorganisms and an inhibitory agent for microbes. *African Health Sciences*, 7(3), pp. 159-165.

Ozkok, A., Sorkun, K. and D'arcy, B.R., 2010. Total phenolic acid and total flavonoid content of Turkish pine honeydew honey. *Journal of ApiProduct and ApiMedical Science*, 2(2), pp. 65-71.

Philip, Y. and Mohd Fadzelly, A.B., 2015. Antioxidative and acetylcholinesterase inhibitor potential of selected honey of Sabah, Malaysian Borneo. *International Food Research Journal*, 22(5), pp. 1953-1960.

Pierce Quantitative Peroxide Assay Kit, 2016. 1st ed. [ebook] pp.1-4. Available at: https://tools.thermofisher.com/content/sfs/manuals/MAN0011275_Pierce_Quant_Peroxide_Asy_UG.pdf [Accessed 10 October 2016].

Pontis, J.A., Costa, L.A.M.A., Silva, S.J.R. and Flach, A., 2014. Color, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. *Journal of Food Science and Technology*, 34(1), pp. 69-73.

Pyrzynska K. and Biesaga, M., 2009. Analysis of phenolic acids and flavonoids in honey. *TrAC Trends in Analytical Chemistry*, 28(7), pp. 893-902.

Qamer, S., Ahamed, F., Ali, S.S. and Shakoori, A.R., 2013. Effect of storage on various honey quality parameters of *Apis dorsata* honey from Nepal. *Pakistan Journal of Zoology*, 45(3), pp. 741-747.

Rahaman, N.L., Chua, L.S., Sarmidi, M.R. and Aziz, R., 2013. Physicochemical and radical scavenging activities of honey samples from Malaysia. *Agricultural Sciences*, 4(5), pp. 46-51.

Rane, M.D. and Doke, K., 2014. Physicochemical properties, antioxidant features of honey samples from ecological niches of western Maharashtra. *International Journal of Science and Research*, 3(8), pp. 1723-1725.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9), pp. 1231-1237.

Rebiai, A., Lanez, T. and Chouikh, A., 2015. Physicochemical and biochemical properties of honey bee products in South Algeria. *Scientific Study and Research*, 16(2), pp. 133-142.

Rossano, R., Larocca, M., Polito, T., Perna, A.M., Padula, M.C., Martelli, G. and Riccio, P., 2012. What are the proteolytic enzymes of honey and what they do tell us? A fingerprint analysis by 2-D zymography of unifloral honeys. *Plos One*, 7(11), pp. 1-17.

Saric, G., Markovic, K., Lez, V., Hruskar, M. and Vahcic, N., 2013. Changes of antioxidant activity in honey after heat treatment. *Czech Journal of Food Sciences*, 31(6), pp. 601-606.

Shafiq, H., Iftikhar, F., Ahmad, A., Kaleem. M. and Sair, A.T., 2014. Effect of crystallization on the water activity of honey. *International Journal of Food and Nutritional Sciences*, 3(3), pp. 1-6.

Shahnawaz, M., Sheikh, S.A., Hussain, M., Razaq, A. and Khan, S.S., 2013. A study on the determination of physicochemical properties of honey from different valley of Gilgit-Baltistan. *International Journal of Agricultural*

Science Research, 2(2), pp. 49-53.

Shalaby, E.A. and Shanab, S.M.M., 2012. Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of *Spirulina platensis*. *Indian Journal of Geo-Marine Sciences*, 42(5), pp. 556-564.

Shalaby, E.A. and Shanab, S.M.M., 2013. Antioxidant compounds, assays of determination and mode of action. *African Journal of Pharmacy and Pharmacology*, 7(10), pp. 528-539.

Singh, M.P., Chourasia, H.M., Agarwal, M., Malhotra, A., Sharma, M., Sharma, D. and Khan, S., 2012. Honey as a complementary medicine. *International Journal of Pharma and Bio Sciences*, 3(2), pp. 1-20.

Sohaimy, S.A., Masry, S.H.D. and Shehata, M.G., 2015. Physicochemical characteristics of honey from different origins. *Annals of Agricultural Sciences*, 60(2), pp. 279-281.

Stark, J.M. and Firestone, M.K., 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Applied and Environmental Microbiology*, 61(1), pp. 218-221.

Sulieman, A.M., Abdelhmied, B.A. and Salih, Z.A., 2013. Quality evaluation of honey obtained from different sources. *Food and Public Health*, 3(3), pp. 137-141.

Umarani, S., Eswaran, V.U., Keerthika, E., Mathumitha, K., Elakkiya, S. and Bhargava, H.R., 2015. A relative study on the chemical composition among the pure and branded honey. *World Applied Sciences Journal*, 33(3), pp. 401-408.

Valachova, I., Bucekova, M. and Majtan, J., 2016. Quantification of bee-derived peptide defensin-1 in honey by competitive enzyme-linked immunosorbent assay, a new approach in honey quality control. *Food Analysis, Food Quality and Nutrition*, 34(3), pp. 233-238.

Voldrich, M., Rajchl, A., Cizkova, H. and Cuhra, P., 2009. Detection of foreign enzyme addition into the adulterated honey. *Czech Journal of Food Sciences*, 27(1), pp. 280-281.

Vorlova, L. and Pridal, A., 2002. Invertase and diastase activity in honeys of Czech provenience. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 50(5), pp. 57-65.

Wearmouth, A., 2015. *Correlation of DHA in nectar and MGO in honey*. [Online] Available at: http://www.kauriparknurseries.co.nz/correlation-dha-nectar-mgo-honey/ [7 January 2017].

Wilczynska, A., 2010. Phenolic content and antioxidant activity of different types of polish honey – a short report. *Polish Journal of Food and Nutrition Sciences*, 60(4), pp. 309-313.

Wood, J.M., 2015. Bacterial responses to osmotic challenges. *Perspectives on: The response to osmotic challenges*, 145(5), pp. 381-388.

Zappala, M., Fallico, B., Arena, E. and Verzera, A., 2005. Methods for the determination of HMF in honey: a comparison. *Food Control*, 16(3), pp. 273.

Zapotoczny, P., Kawałko, T. and Bakier, S., 2010. Determination of the physical characteristics of food raw materials by spectrophotometry – the example of honey. *Technical Sciences*, 2010(13), pp. 43-45.

Zerrouk, S.H., Fallico, B.G., Arena, E.N., Ballistreri, G.F. and Boughediri, L.A., 2011. Quality Evaluation of some honey from the central region of Algeria. *Jordan Journal of Biological Science*, 4(4), pp. 245-248.

turnitin **Turnitin Originality Report**

FYP Thesis Draft by Choi Wan Ng

From FYP Thesis Draft (FYP OCT 2016)

Processed on 02-May-2017 00:27 MYT
ID: 797697460
Word Count: 16916

Similarity Index	Similarity by Source	
15%	Internet Sources:	10%
	Publications:	12%
	Student Papers:	7%

sources:

1 1% match (Internet from 30-Aug-2016)
https://ccj.springeropen.com/articles/10.1186/1752-153X-7-138

2 1% match (publications)
Abachi, Mouayed Q. Al Hadi, Hind. "Simple, rapid and sensitive method for the determination of mefenamic acid in pharmaceutical prepara", Journal of Analytical Chemistry, August 2014 Issue

3 1% match (Internet from 31-Dec-2015)
http://bmccomplementalternmed.biomedcentral.com/articles/10.1186/1472-6882-13-43

4 1% match (publications)
Sime, D., M. Atlabachew, M. R. Abshiro, and T. Zewde. "Total phenols and antioxidant activities of natural honeys and propolis collected from different geographical regions of Ethiopia", Bulletin of the Chemical Society of Ethiopia, 2015.

5 < 1% match (publications)
Saxena, S.. "Physical, biochemical and antioxidant properties of some Indian honeys", Food Chemistry, 20100115