INFLUENCE OF ADDITION OF GINGER ON SENSORY QUALITY, PHYSICOCHEMICAL PROPERTIES AND BIOACTIVITIES OF MALAYSIAN MULTIFLORAL HONEY

By

YIP SEE CHENG

A project report submitted to the Department of Allied Health Sciences

Faculty of Science

Universiti Tunku Abdul Rahman

in partial fulfilment of the requirements for the degree of

Bachelor of Science (Hons) Biomedical Science

May 2022

ABSTRACT

INFLUENCE OF ADDITION OF GINGER ON SENSORY QUALITY, PHYSICOCHEMICAL PROPERTIES AND BIOACTIVITIES OF MALAYSIAN MULTIFLORAL HONEY

YIP SEE CHENG

Honey and ginger are functional food with therapeutic values. Although their mixture is believed to exhibit various health benefits, scientific information on honey-ginger mixture is very limited. Therefore, this study aimed to investigate the influence of added Bentong ginger on sensory quality, physicochemical properties and bioactivities of multifloral honey produced by *Apis cerena* bee. In this study, the appearance, taste, aroma, texture and acceptability of ginger honey and base honey were assessed in sensory analysis. Physicochemical parameters evaluated were colour intensity, acidity, sugar content, moisture content, water activity and electrical conductivity. Also, antioxidant capacity of honey was measured using total phenolic content, hydroxyl radical scavenging activity, superoxide anion scavenging activity and iron chelating activity while antibacterial ability of honey was examined using agar well diffusion method. Results showed that ginger honey scored similarly to base honey for appearance, texture and most of the sensory attributes, except for the increased woody, chemical and spoiled but decreased

sweetness, floral-fruity and warm attributes. However, the base honey was better accepted by the panelists. The colour intensity, pH, electrical conductivity and reducing sugar content of ginger honey (268.0-304.5 mAU; 4.48-4.88; 444.7-532.3 µS/cm; 59.64-61.92 %, respectively) were significantly higher than base honey (206.0 mAU; 4.53; 290.0 µS/cm; 57.12 %, respectively). Ginger honey also showed significant higher total phenolic content, superoxide anion scavenging activity and iron chelating activity (37.08-45.95 mg GAE/kg; 80.74-85.55 %; 13.32-21.28 %, respectively) than base honey (20.19 mg GAE/kg; 56.27 %; 1.64 %, respectively). All honey samples inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* but no significant difference was found between their antibacterial capacities. Generally, addition of ginger into honey changed its physicochemical properties, taste and aroma but reduced its acceptability. The added ginger also enhanced the antioxidant ability but had no significant impact on the antibacterial capacity of honey.

ACKNOWLEDGEMENT

First of all, I would like to express my sincere thankfulness to my final year project supervisor, Mr. Ng Wen Jie for offering me a great opportunity to participate in this project. His appreciated guidance, support and supervision throughout the project helped me a lot in completing this final year project successfully. Moreover, I would like to convey my deep gratitude to my co-supervisor, Dr. Ee Kah Yaw for his patient assistance and advice that assisted me to improve my project design.

Additionally, I would like to express my sincere gratitude to my beloved bench mate Chin Hui Yee, Chu Mei Huan and Liong Xin Yee for their support and cooperation in the whole project timespan. Also, I would like to thank the lab officers who are always willing to assist me during the benchwork especially Mr. Saravanan a/l Sivasangaran, Ms. Fatin Masturah Mohd Isa and Ms. Natrah Binti Khalid.

Lastly, I would like to express my thanks to my friends and family members for motiving me to complete my project.

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 any other degree at UTAR or other institutions.

YIP SEE CHENG

APPROVAL SHEET

This project report entitled **"INFLUENCE OF ADDITION OF GINGER ON SENSORY QUALITY, PHYSICOCHEMICAL PROPERTIES AND BIOACTIVITIES OF MALAYSIAN MULTIFLORAL HONEY"** was prepared by **YIP SEE CHENG** and submitted as a 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)

Supervisor

Department of Allied Health Sciences

Faculty of Science

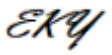
Universiti Tunku Abdul Rahman

Date: 27/5/2022

APPROVAL SHEET

This project report entitled **"INFLUENCE OF ADDITION OF GINGER ON SENSORY QUALITY, PHYSICOCHEMICAL PROPERTIES AND BIOACTIVITIES OF MALAYSIAN MULTIFLORAL HONEY"** was prepared by **YIP SEE CHENG** and submitted as a partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Biomedical Science at Universiti Tunku Abdul Rahman.

Approved by:



(Mr. EE KAH YAW) Date: 22 April 2022

Co-Supervisor

Department of Agricultural and Food Science

Faculty of Science

Universiti Tunku Abdul Rahman

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: 22 April 2022

Permission Sheet

It is hereby certified that **YIP SEE CHENG (ID: 18ADB03172)** has completed this final year project entitled "INFLUENCE OF ADDITION OF GINGER ON SENSORY QUALITY, PHYSICOCHEMICAL PROPERTIES AND BIOACTIVITIES OF MALAYSIAN MULTIFLORAL HONEY" under the supervision of Mr. Ng Wen Jie (Supervisor) from the Department of Allied Health Sciences, Faculty of Science, and Dr. Ee Kah Yaw (Co-Supervisor) from the Department of Agricultural and Food Science, Faculty of Science.

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

Yours truly

(YIP SEE CHENG)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOAC	Association of official analytical collaboration
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
a_w	Water activity
CFU	Colony-forming unit
°C	Degree Celsius
DNSA	3,5-dinitrosalicylic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
et al.	*et alia*
GAE	Gallic acid equivalence
g/mL	Gram per milliliter
HPLC	High performance liquid chromatography
µg	Microgram
µg/mL	Microgram per milliliter
µL	Microliter
µM	Micromolar
µS/cm	MicroSiemens per centimeter

M	Molarity
mAU	Milli-absorbance unit
mg/kg	Milligram per kilogram
mg/mL	Milligram per milliliter
MH	Mueller-Hinton
mL	Milliliter
mM	Millimolar
ppm	Parts per million
% RSA	Percentage radical scavenging activity
PBS	Phosphate-buffered saline
pH	Potential of hydrogen
rpm	Revolutions per minute
ROS	Reactive oxygen species
spp.	Species
TBA	Thiobarbituric acid
TPC	Total phenolic content
v/v	Volume per volume
w/v	Weight per volume

CHAPTER 1

INTRODUCTION

Honey is a natural sweetener produced by honey bees, with a good acceptance by consumers due to its taste qualities, health benefits and nutritional values (Aleksandra et al., 2017). The main constituents in honey are glucose and fructose, followed by small amounts of other important constituents such as enzymes, vitamins, water, flavonoids, phenolic acids, amino acids, proteins, and minerals (Bertoncelj et al., 2007). Different types of honey have different compositions due to the variation in their geographical location, floral origin and the influence of various external factors, including weather, environment and handling technique (Moniruzzaman et al., 2013).

Spices are food additives that have been commonly used in food processing because of their unique taste and aroma. One of the worldwide well-known spices is ginger (*Zingiber officinale* Roscoe). Ginger is an herbaceous plant with intense flavour and aroma, making it become a popular flavouring agent and medicine in Malaysia for centuries. Approximately 100 components have been found in ginger, the major compounds isolated from ginger are zingerone, shogaols, zingiberene and gingerols, followed by other minor compounds such as vitamins, terpenes and minerals (Anh et al., 2020).

Honey and ginger are therapeutic agents in different cultures since ancient times. Both of them are rich sources of antioxidant and antimicrobial compounds. Honey consists of flavonoids, aromatic aldehydes and phenolic compounds so it is able to scavenge free radicals and inhibit bacteria (Martos et al., 2008). As a result, the role of honey has been extended to the treatment of inflammation, cold, skin wound and cardiovascular disorder. The gingerols in ginger not only gives it a pungent aroma, but is the primary component that exhibit antioxidant, antimicrobial, and anti-inflammation effects. The zingerone is usually yielded from gingerols during heating or drying process and it further enhances the theraupeutic effects of ginger. Therefore, ginger may be used in relieving nausea, vomiting, cough, constipation and fatigue (Anh et al., 2020).

Recently, due to increasing interest in consuming food with health values, products based on the addition of spices such as ginger in honey have been marketed in Malaysia intending to combine the beneficial properties of honey and ginger. The enhancing effect of added ginger extract on the bioactivities of honey has been reported. Although some research findings outlined the addition of ginger had no significant effect on the antimicrobial activity of honey, several studies had showed the mixtures have increased phenolic antimicrobial content and radical scavenging activities (Ewnetu et al., 2014; Aleksandra et al., 2017; Bernard et al., 2021). However, there are insufficient studies that evaluate the influence of the addition of ginger extract on the properties of Malaysian multifloral honey in these four

aspects which are sensory, physicochemical, antimicrobial and antioxidant properties.

Hence, the objectives of the current study were:

i. To evaluate the influence of the addition of ginger to the Malaysian multifloral honey on sensory quality, physicochemical properties, antioxidant and antibacterial activities.
ii. To compare the sensory quality, physicochemical parameters and bioactivities between honeys with different concentrations of ginger extract.

CHAPTER 2

LITERATURE REVIEW

2.1 Honey

2.1.1 Introduction

Honey is a natural food product with a sweet taste, thick texture and dark yellowish appearance (Ng et al., 2014). The previous study has reported that natural honey contains around 200 substances. The major compositions of honey are glucose and fructose while other substances such as gluconic acid, acetic acid, flavonoids, ascorbic acid, niacin and polyphenols are also present in a small amount (Eteraf-Oskouei and Najafi, 2013). The taste, aroma and appearance of honey depend on the type of plants that the bees feed (Meo et al., 2017). The physical and chemical characteristics of honey also are affected by its floral origin, entomological source, geographical origin, climatic circumstances, environmental dynamics and processing condition (Kadri et al., 2016).

The honey bees use nectar to produce honey. Nectar is a sugary liquid generated by flowers to attract insect pollinators. As shown in Figure 2.1, the scent chemicals in nectar draw honey bees to the flowers and the honey bees use their hollow proboscis to suck out nectar from the flowers. The nectar is stored in the proventriculus, the

first chamber of their stomach. The invertase in the proventriculus digest sucrose into glucose and fructose. Then, the honey bees travel back to their hive and pass the nectar to other bees via regurgitation to reduce the water level of the nectar. The regurgitation stopped when the viscous nectar is deposited onto a honeycomb. The honey bees fan the honeycomb with their wings to evaporate the nectar. Finally, the honey bees seal the honeycomb with their abdomen secretion to solidify the honeycomb into beeswax (Life Science, 2013; Villazon, 2021).

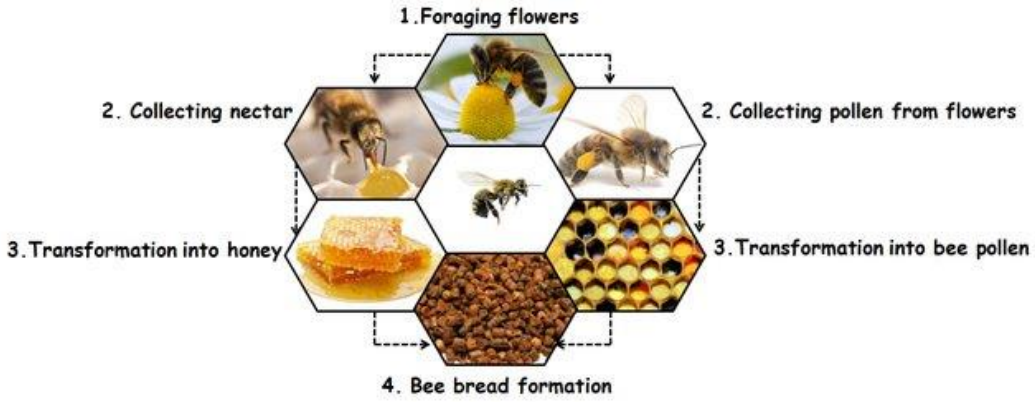


Figure 2.1: The process of honey production (Bakour et al., 2022).

2.1.2 Honey in Malaysia

Malaysia is a tropical country with rich flora and fauna resources. There are several honey producing bees available in Malaysia such as *Apis dorsata* (forest bee), *Apis mellifera* (Australian honey bee) and *Trigona thorasica* (stingless bee). Malaysian honey is typically produced in Johor, Sabah, Melaka and Sarawak. The beekeeping industry is one of the important agricultural sectors in Malaysia. According to

Saludin et al., (2019), there are around 30 metric tonnes of honey production per year by 750 to 1,000 beekeepers in Malaysia.

Apis cerana (as shown in Figure 2.2*)* is one of the local honey bee species in Malaysia widely used for honey production. *Apis cerana*, also known as Eastern hive-bee, is a smaller-sized honey bee, mainly located in East Malaysia. However, due to the high productivity of *Apis mellifera* (as shown in Figure 2.3*)* in modern breeding, it has been hosted into many *Apis cerana* habitats and causing the reduction of *Apis cerana* populations. In Asian markets, *Apis cerana* honey is usually three to five times more expensive than *Apis mellifera* honey because of its limited production and local customer preferences. Some dishonest beekeepers may mix *Apis mellifera* honey into *Apis cerana* honey to earn an extra profit (Zhang et al., 2019).

Figure 2.2: View of *Apis cerana* honey bee (Lichtenstein et al., 2019).

Figure 2.3: View of *Apis mellifera* honey bee (Lichtenstein et al., 2019).

Multifloral honey in Malaysia is mainly produced by *Apis cerana* bees which collect nectar from more than one type of flower (Moniruzzaman et al., 2013). A previous study by Taormina (2001) outlined multifloral honey as amber-coloured honey with a creamy appearance and unique flavour of various flower nectar. Its bioactive compounds contributed to its antioxidant and antimicrobial ability. These compounds include catalase, ascorbic acid, phenolic acids and flavonoids, Furthermore, the therapeutic role of multifloral honey is relatively credited to its antioxidant properties.

2.2. Ginger

2.2.1 Introduction

Ginger, an underground rhizome of *Zingiber officinale*, is one of the most widely used ingredients in cooking and medicine (as shown in Figure 2.4*)*. Besides, ginger

has been used as a natural preservative and stabilizer during food processing due to its irreplaceable aroma and flavour. A food preservative is an agent which hinders, slows down or halts the growth of microbes on food to prolong its shelf life and avoid food spoilage (Delores et al., 2004). The high amount of citric acid in ginger, together with carbon dioxide can protect food from microbial deterioration because the low pH and lack of oxygen environment. This making ginger an acid preservative for food and drink (Glevitzky et al., 2009). Natural preservatives like ginger are safer than chemical preservatives so they are a more preferable choice for consumers to minimize the possible side effects of synthetic chemical preservatives (Mishra and Behal, 2010).

Figure 2.4: View of *Zingiber officinale* Roscoe (Gupta and Sharma, 2014).

Ginger is an unfertile species. It cannot be sexually propagated because it needs rhizomes for vegetative propagation. The rhizome of ginger is usually the starting material for its cultivation. On the other hand, plant diseases such as leaf spot, bacterial shrivel, leaf yellowing and delicate decay can be rapidly spread through

the fragmentation of ginger rhizomes during vegetative reproduction. In addition, the high demand for ginger rhizomes as planting material causes the rise of its cost (Kasilingam et al., 2018).

Ginger has been reported to have hypoglycaemic, anti-inflammatory, antioxidant and antimicrobial activities *in vitro* and *in vivo* studies. Thus, ginger has been used as a traditional medicine in many parts of the world. According to Fletcher (2020), enzymes in ginger can help the body to reduce the gas that forms in the intestinal tract during digestion and increase the digestive tract movement to relieve constipation. Ginger can also alleviate morning sickness and chemotherapy-induced nausea. However, the use of ginger as a medicine has not been well investigated because many compounds in ginger have not been well studied so the healing quality of ginger is not guaranteed (Sahdeo and Amit, 2015). Hence, the patients who wish to take ginger as a supplement are advised to consult their healthcare provider first because the interaction of ginger with other medications may induce other health complications.

2.2.2 Ginger in Malaysia

Malaysia is a famous ginger-producing country in Southeast Asia. About 9.017 metric tons of ginger rhizomes are yielded per year in Malaysia (Nafi et al., 2014). In Malaysia, gingers are mostly produced in Bentong of Pahang, Bakun of Sarawak,

Keningau and Tambunan of Sabah. Besides, to fulfill the high demand for ginger in Malaysia, the entrepreneurs also import gingers from China and Indonesia.

Bentong ginger (*Zingiber officinale* Var. Bentong), only cultivated at Bentong highland, is the most popular variety of *Zingiber officinale* in Malaysia. As shown in Figure 2.5, it has a bigger rhizomes and lower fibrous pulp than other gingers produced in Malaysia. Most of Bentong gingers are planted in mountain slopes like Janda Baik and Bukit Tinggi, Bentong, Pahang. Second phase planting of ginger on the same location needs at least 6 years interval and no commercial crop can be carried out on the land in between. The demanding requirement of the cultivation area results in the low production and high cost of this high-quality ginger. Furthermore, the ginger crops are easily destroyed by various soil-borne diseases (Suhaimi, Mohamad and Hani, 2014).

Figure 2.5: View of Bentong ginger (Alagesh, 2019).

Similar to other gingers, the leaves, stem and rhizome of Bentong ginger contain high content of flavonoids and polyphenols, including flavones glycosides, rutin, beta-carotene, ascorbic acid and terpenoids. These natural antioxidant compounds are associated with the pharmacological effect of Bentong ginger. The ginger rhizome extract is also a rich source of antimicrobial substances such as citral, citronellal and linalool (Ghasemzadeh et al., 2010). The quality of Bentong ginger is better when compared to gingers from China, Indonesia, Thailand, Taiwan and the United States because of its special phenolic profile, at which the Bentong ginger contains higher level of beta-citronellol, oxygenated monoterpenes and linalool than the non-Bentong gingers (Syuhaidah, 2020). Because of all these health advantages, the demand for Bentong ginger is highly increased in the local and international markets (Nafi et al., 2014).

2.3 Sensory Properties

According to Heredia et al. (2013), sensory properties of food products, mainly texture, appearance, aroma, taste and acceptability are perceived by the five primary human senses— tactile, visual, olfactory, gustatory, auditory and common chemical sense. The appearance and the smell of a food product are the major qualities that attract consumers while the taste that combines both oral and nasal stimulation is the largest factor that determines its acceptability. Unlike features such as nutritional value, ingredients and safety that are uncontrollable by consumers, sensory properties are directly received by the consumers and

immediately influenced their perception. In addition, the acceptability of consumers is very important for the food industry to control the organoleptic characteristics of their food products.

The sensory properties of a food product can be assessed by sensory analysis. The human tongue can differentiate five qualities of taste, which are sweetness, bitterness, sourness, saltiness and savoriness while the human nose can differentiate a huge number of volatile compounds. Sensory analysis can be used in many fields, especially in the food industry to build up the organoleptic profile of different products and estimate how well the products are perceived by the consumers. Until the 1960s, sensory analysis purely relied on the personal experience of assessors, making this method simple but lack of reproducibility. In the twentieth century, an improved sensory evaluation technique was developed by using assessor panels, controlled experimental settings and statistical systems to process the results. The improved methods yield reproducible results. However, the complexity and high costs of the new methods are the limitations for the routine use in quality control (Piana et al., 2004).

The first sensory analysis of honey was held in France by the Gonnet team and the use of sensory analysis for honey inspired other researchers in Italy. They contributed many efforts in training the panelists, inducing the formation of the

National Register of Experts in the Sensory Analysis of Honey to set up a standardized methodology for the analysis (Piana et al., 2004). The guidelines for evaluation forms, tasting methods, assessor selection and sensory descriptions were established so the sensory analysis of honey can be carried out in a more organized way (Persano Oddo et al., 2000). In 1990, International Honey Commission (IHC) was created in order to design a set of honey standards that are applicable worldwide for routine honey analysis including sensory analysis (Piana et al., 2004).

For honey, sensory analysis helps to discriminate the geographic origin, botanical origin of the honey and to identify its characteristics, including impurities, fermentation and off-odours. Although many characteristics of honey can be evaluated by laboratory methods, there are still some characteristics that are currently only can be determined via sensory analysis such as the presence of unique taste and odour attributes (Moumeh et al., 2020). With those detailed properties, the honey manufacturers can define their honey product standards according to botanical denominations as well as other labels. Moreover, sensory evaluation is very important in confirming the conformity of unifloral honey as it can disclose the presence of botanical components that are not recognized by other analytical systems (Oddo and Piro, 2004).

2.4 Physicochemical Properties

2.4.1 Introduction

Physicochemical parameters are the most important determinants for the quality of honey. The physicochemical properties of honey are generally explained as its physical and chemical characteristics (National Chemical Emergency Centre, 2022). The example of physicochemical properties are colour intensity, pH, water activity, moisture content, electrical conductivity, total and reducing sugars content (Moniruzzaman et al., 2013). The physicochemical properties of honey can be affected by its geographical origin and climate factors (Kadri et al., 2016).

Honey is an energy provider because about 85 % of honey is made up of high-energy carbohydrates while the major sugar contents in honey are easily digestible fructose and glucose (Rahman et al., 2010). Furthermore, the glucose, fructose and water ratio in honey is another important element that determines honey quality because these ratios determine the tendency of granulation (Dobre et al., 2012; Buba et al., 2013). The Codex Alimentarius Commission (2001) and International Honey Commission established the set of standards for the physicochemical properties of honey and these standards allow the honey manufacturers as well as the researchers to determine whether a honey is in good quality or not.

2.4.2 Colour Intensity

The honey colour is rated based on the standards posted by USDA (2017). The colour of bee honey normally varies from light yellow to amber as well as dark amber. Black, green or red coloured honey only appeared in rare cases (Bogdanov et al., 2008). Changes in colour might be caused by the intervention of beekeepers, use of old wax combs, high temperatures, light stimulation, minerals content and heavy metal contamination (El-Metwally, 2015).

The colour intensity of honey is revealed by ABS_{450}, at which the absorbance of honey is measured at 450 and 720 nm while the colour intensity is represented by the net absorbance in the unit of mAU (Moniruzzaman et al., 2013). ABS_{450} can detect the presence of carotenoids and flavonoids in honey. In addition, the honey colour is directly associated with the polyphenols content in honey, at which darker honey commonly consists of a higher amount of phenolic compounds. Those pigments and phenolic compounds contribute to the antioxidant properties of honey (Al-Farsi et al., 2018).

2.4.3 Acidity

Honey is acidic in nature. The normal pH of honey ranged from 3.53 to 4.03 (Moniruzzaman et al., 2013). The pH value of honey can indicate its freshness. This is because fermentation of sugars into organic acid in fresh honey is lesser when compared to honey that is stored for a long time, thus the acidity of fresh honey is

lower. The honey with a pH value ranging from 3.4 to 6.1 is considered fresh honey. The fermentation of sugars also correlated to other two important parameters of honey, which are flavour and stability against microbial spoilage (Bogdanov et al., 2008). The osmotolerant yeasts induce the fermentation of fructose and glucose in honey, leading to the production of ethyl alcohol and carbon dioxide. The alcohol will then further oxidized into acetic acid, causing a sour taste (Khalil et al., 2012).

The main organic acid formed from honey fermentation is gluconic acid. It is generated via enzymatic digestion of glucose (Olaitan et al., 2007). The presence of gluconic acid in honey is mainly attributed to its low pH. The low pH of honey help in hindering microbial growth and prolonging the shelf life of honey (Mandal and Mandal, 2011). In addition, the acidity of honey can stimulate wound healing by reducing protease activity, enhancing oxygenation, inducing macrophages and fibroblast to act on the injured site (Minden-Birkenmaier and Bowlin, 2018).

2.4.4 Water Activity

Honey is a supersaturated sugar solution. The amount of free water in honey is defined as its water activity (a_w). However, the strong interaction between the sugar molecules limits the concentration of water molecules in honey. Therefore, the water activity in honey usually varies from 0.5 to 0.65 (Abramovic et al., 2008). The extent of free water is an important feature for moisture migration and microbial stability of honey as the osmophilic yeasts only require 0.6 as their

minimal water activity for growth (Gleiter et al., 2006). At the same time, the water activity of honey also linearly correlated with its moisture content (Zamora et al., 2006).

In general, many factors affect the water activity in honey, including the type of honey, the physical state of honey, filtration of honey and storage condition (Yap et al., 2019). The dehydration process can decrease the water activity of honey to a safe level that prohibits microbial growth. The storage of honey in an environment with low humidity lessens its water activity too. Moreover, heating process also reduces the water activity of honey and water activity influences the honey crystallization, at which the low water activity in honey speeds up its crystallization (Wilczyńska and Ruszkowska, 2014).

2.4.5 Electrical Conductivity

Electrical conductivity is a good criterion to determine the botanical origin and authentication of honey, especially the differentiation between floral and honeydew honey (Adenekan et al., 2010). This is because the floral honey has a lower electrical conductivity than the honeydew honey, except for manuka honey, tea tree honey and eucalyptus honey (Codex Alimentarius Commission, 2001). Therefore, electrical conductivity replaced ash content to become one of the parameters for routine honey control (Adenekan et al., 2010). The measurement of electrical conductivity depends on the acid and minerals like ash in honey. The acid and

mineral content can dissociate into ions and then conduct electricity in an aqueous solution. Thus, they showed a linear relationship with the electrical conductivity of honey, at which the higher the acid and mineral content, the higher the electrical conductivity of honey (Baloš et al., 2018).

The presence of heavy metals in honey could raise the electrical conductivity so the Codex Alimentarius Commission (2001) stated that the electrical conductivity of honey bee honey should be lower than 0.8 mS/cm to verify the absence of the heavy metal in the honey. Furthermore, the electrical conductivity varies according to the amount of plant pollen. Honey with less pollen shows lower ability in conducting electricity (Fredes and Montenegro, 2006).

2.4.6 Total Sugar Content

More than 90 % of the total dry weight of honey is made up of sugar. There are at least 25 different kinds of sugar in honey, the examples of them are sucrose, glucose and fructose (Weston and Brocklebank, 1999). The total sugar content is highly linked to the botanical source and degree of maturity of honey. Sucrose level of honey that more than 5 % is considered high level. Early harvested honey usually shows a high level of sucrose due to the incomplete invertase - catalyzed conversion of sucrose into reducing sugars (Belay et al., 2017). The viscosity and hygroscopicity of honey are also mainly affected by its total sugar content, at which

the honey with high total sugar content is more viscous than the honey with low total sugar content (Kamal and Klein, 2011).

Honey with high total sugar content is normally associated with high osmotic pressure and low moisture content. Those characteristics can impede the formation of 5-hydroxymethylfurfural from sugars and inhibit the growth of microbes. In other words, honey with high total sugar content has longer shelf life than honey with low sugar content. Additionally, the total sugar content can affect the flavour of honey as honey with high total sugar content has a sweeter taste (Moniruzzaman et al., 2013).

2.4.7 Reducing Sugar Content

The dominant sugar types in honey are the reducing sugars, glucose and fructose, which constitute about 95 % of the total sugars in honey (Ajibola, 2015). The Codex Alimentarius Commission (2001) mandates that the amount of reducing sugars in honey should be more than 60 %.

Granulation of honey is depending on its glucose content, at which honey with a glucose content lesser than 30 % granulate slowly (Manikis and Thrasivoulou, 2001). At the same time, the time required for the honey to granulate is determined by its glucose to water content ratio. Honey with glucose to water ratio less than or

equal to 1.7 is non-granulating honey whereas honey with ratio more than or equal to 2.1 is rapid granulating honey (Kaakeh and Gadelhak, 2005; Dobre et al., 2012). Similarly, the fructose to glucose ratio is a critical parameter to explain honey granulation because fructose is more water-soluble than glucose so it can reduce the tendency of granulation (Escuredo et al., 2014). Incomplete granulation creates an advantageous environment for yeast to carry out fermentation because the crystalline layer is overlapped by liquid honey with high water content that promotes fermentation (Escuredo et al., 2013).

2.4.8 Moisture Content

Moisture content is defined as the total amount of water available in honey. It is an important indicator of honey quality (Moniruzzaman et al., 2013). Based on the standard approved by Codex Alimentarius Commission (2001), the moisture content of honey bee honey should be less than 20 %. In the honey industry, the moisture content is always confused with the water activity because both of them are involved in the fermentation. Moisture content referred to the water content in honey while the water activity referred to the excess amount of water in honey that reacts with microorganisms. Even they showed a positive correlation with each other, these two parameters are measured for different reasons. Moisture content is important to estimate the total sugar of honey whereas water activity is useful in setting an optimal storage condition.

In general, the low moisture content is the characteristic of good quality honey because low water content can avoid microbial fermentation and granulation during storage. The lower the moisture content, the lower probability for the honey fermentation to occur during storage. This is because the microorganisms need water to carry out their biological activity and produce several by-products such as acid and toxins that destroy food. Hence, the honey can have a longer shelf life during storage (Terrab et al., 2003). Overall, the low moisture content indicates the good storage ability of honey. However, the moisture content in honey relies on the humidity and temperature of its geographical origin. Furthermore, the moisture content of honey also depends on the harvesting and storage management of honey (Olaitan et al., 2007).

2.5 Antibacterial Properties

2.5.1 Antibacterial Effect of Honey

Natural honey has been proven to show a broad-spectrum antibacterial activity against several bacteria, including the wound-infecting *Escherichia coli* and *Staphylococcus aureus* (Mandal and Mandal, 2011). Since honey is a high viscosity concentrated sugar solution, it has low water activity and low moisture content. The reduced humidity environment in honey inhibits bacterial growth and thus giving honey an antibacterial ability (Molan, 1992). The antibacterial characteristics of honey are also contributed by its low pH, hydrogen peroxide and high osmolarity (Mavric et al., 2008). Sugar fermentation that occurred naturally in honey generates

organic acids such as gluconic acid, resulting in the low pH value of honey (Abselami et al., 2018). These acids create an environment that is unfavourable for bacterial survival so the growth of bacterial pathogens is inhibited (Koochak et al, 2010). On the other hand, the low pH denatured the enzyme and altered the ionic charges to stop the metabolism of microbes (Blamire, 2000).

Moreover, the presence of hydrogen peroxide in honey is its main antibacterial agent. The hypopharyngeal gland of honey bees produces glucose oxidase which oxidizes glucose into gluconic acid and hydrogen peroxide (Tao et al., 2009). Hydrogen peroxide forms hydroxyl radicals which can induce the peroxidation of cellular nucleic acids, proteins and lipids (Zhou et al., 2009). Consequently, the viability of cells is reduced and eventually died (Brudzynski et al., 2012). Furthermore, the phenolic compounds in honey show antibacterial activity too. Based on a previous study of Estevinho et al. (2008), flavonoids and gallic acids in honey are effective in prohibiting the growth of numerous bacterial species, including *Staphylococcus aureus, Staphylococcus lentus, Escherichia coli* and *Bacillus subtilis.*

2.5.2 Antibacterial Effect of Ginger

Ginger consists of a range of chemicals that come with an antibacterial effect, including terpenes and phenolic compounds. These antimicrobial components are mainly found in ginger rhizomes. Terpenes in ginger are β-sesquifelenolene, β-

bisabolene, zingiberene, α-farnesene and α-curcumene whereas phenolic compounds in ginger are shogaol, gingerol and paradols (Beristain-Bauza et al., 2019). Among those antibacterial components, gingerols and shagaol are the major contributors that inhibit the replication of bacteria (Nychas and Skandamis, 2003). Besides, the study conducted by Alzoreky and Nakahara (2003) revealed the ability of ginger in reducing the growth of a wide range of Gram-negative and Gram-positive bacteria such as *Escherichia coli, Salmonella infantis, Staphylococcus aureus* and *Bacillus cereus*.

However, the inhibition of bacterial growth by ginger is active at high concentration and inactive at very low concentrations so the antibacterial activity of the ginger extract is dose-dependent (Malu et al., 2009). Furthermore, the antibacterial effect of ginger is significantly affected by its genetic composition, growing environment, collecting condition and extraction methods. A previous study also found that Gram-positive bacteria were more sensitive to the antibacterial activity of ginger because ginger usually target on the thick peptidoglycan layered cell wall of Gram-positive bacteria to exert its antibacterial effect (Burt, 2004).

2.5.3 Antibacterial Assays

There are a number of methods available to evaluate the antibacterial efficacy of natural products. Agar well diffusion assay is a widely used antibacterial assay because of its simplicity and reproducibility although this method does not allow

distinguishing between bactericidal and bacteriostatic effects (Valgas De Souza and Smânia, 2007).

Agar well diffusion method begins with inoculating a volume of the microbe over the entire agar surface. Next, holes will be punched aseptically using a sterile cork borer on the agar so the antimicrobial agent can be introduced into the wells. After overnight incubation, the antimicrobial agent is diffused into the agar medium and the researchers can examine how well the antimicrobial agent inhibits tested microbial growth (Balouiri et al., 2016).

The agar that is usually used in agar well diffusion assay is Mueller-Hinton (MH) agar due to its non-selectivity. This agar can support the growth of almost all bacteria and allow the diffusion of antimicrobial agent. The starch in this agar can also absorb toxins released by bacteria so the result of the tested antimicrobial agent will not be influenced by any toxins. Thus, the results are more accurate and reliable (Aryal, 2018).

Furthermore, agar well diffusion method is a popular method in evaluating the antibacterial activity of food because this assay allows the food sample to contact directly with bacteria and various samples can be tested at the same time (Zainol et al., 2013). Study of Nayaka et al. (2020) applied this method to assess the inhibition effect of ethanolic honey extracts with different concentrations on bacteria while

study of Gonelimali et al. (2018) used this method to measure the antimicrobial properties of ginger extracts against food pathogens.

2.6 Antioxidant Properties

2.6.1 Antioxidant Effect of Honey

According to Thannickal and Fanburg (2000), reactive oxygen species (ROS) are by-products of cellular metabolism and they are mostly found in the mitochondria. Aerobic energy metabolism requires oxygen to generate ATP because the molecular oxygen acts as the final electron acceptor for cytochrome-c oxidase and is then catalyzed into water via four-electron reduction. Some partially reduced and highly reactive metabolites are produced during electron transfer, including superoxide anion and hydroxyl radicals. When the production of ROS exceeds the antioxidant capacity of a cell, ROS will destroy the cellular macromolecules such as lipids, proteins and nucleic acids. This condition is called oxidative stress and contributed to various pathogenesis of human diseases, comprising cancer, kidney disease and cardiovascular disease.

Antioxidants are substances that can stabilize and scavenge the free reactive radicals to reduce the harmful effect of oxidative stress. Honey exhibits strong antioxidant activity. The unique antioxidant initiators of honey are phenolic acids, vitamin E, vitamin C, peroxidase, catalase and other trace elements (Gheldof et al., 2002). Flavonoids with hydroxyl functional groups can slow down the oxidation

rate too (Martos et al., 2008). The amount of these components in honey varies broadly according to its floral origin, handling and storage method (Wang et al., 2004). Additionally, the amount of antioxidant agents in honey can be indicated by the colour of the honey, at which dark-coloured honey is proven to possess high levels of antioxidant agents (Chang et al., 2002).

Apart from the direct antioxidant activity, researchers also disclosed that the indirect action of honey enhanced the synthesis and activity of antioxidant components like vitamin C, glutathione reductase, beta-carotene and uric acid in the human body (Al-Waili, 2003). The exact mechanisms for the antioxidant activity of honey still need future exploration but the possible mechanisms involved are hydrogen donation, free radical sequestration, metal ion chelation and superoxide radical scavenging (Al-Mamary et al., 2002).

2.6.2 Antioxidant Effect of Ginger

DNA damage may happen if there is an imbalance between free radical production and removal so extra antioxidant supplementation through dietary uptake is important in these circumstances (Ramaa et al., 2006). Ginger is a natural antioxidant that can strengthen the body's defenses because the antioxidant compounds such as geranial, α -zingiberene, β -bisabolene, geraniol, camphene, β -phellandrene and nerol found in ginger were evidenced to reduce the oxidative stress generated by reactive oxygen species (Przygodzka et al., 2014). The

polyphenol components in the ginger allow it to donate its hydrogen atoms and capture the free radicals. Shogaol in ginger can significantly reduce lipid peroxidation and increase the levels of antioxidant enzymes with the help of serum glutathione (El-Sharaky et al., 2009). Furthermore, ginger displayed a therapeutic value in ethanol-induced hepatotoxicity as it can regulate the catalase, glutathione reductase, superoxide dismutase, glutathione peroxidase and glutathione content in the liver tissue (Mallikarjuna et al., 2008). Similar findings were also mirrored in the study of Ghasemzadeh et al. (2010) which validated the medicinal potential of the ginger leaves and rhizome.

As shown in the study of Stoilova et al. (2007), ginger was a very good scavenger for various radicals, including hydroxyl radicals and 2,2-Diphenyl-1-picril hydrazyl radical (DPPH). The radical scavenging effect of ginger was even comparable with the synthetic antioxidant so it is a high potential natural preservative for the food industries to protect the food against oxidation. Moreover, the addition of ginger extract in other functional food like honey has been proven to induce changes in the antioxidant efficacy of the honey. In general, the addition of ginger strengthens the antioxidant activity of honey. The more ginger was added, the greater the radical scavenging activity (Aleksandra et al., 2017).

2.6.3 Antioxidant Assays

There are many antioxidant assays available to measure the antioxidant capabilities of natural products. The commonly used assays are total phenolic compounds, hydroxyl radical scavenging activity, superoxide anion scavenging activity and iron chelating activity.

Folin-Ciocalteu assay is a colourimetric assay that measures the total phenolic content in the tested substance. Folin-Ciocalteu reagent consists of phosphomolybdic acid complexes and these complexes react with the hydroxyl functional group of phenolic compounds, forming a blue-coloured phosphotungstic-phosphomolybdenum complex (Bioquochem, 2019). Thus, the concentration of phenolic compounds is directly proportional to the absorbance of the honey-reagent mixture at 725 nm.

The hydroxyl radical scavenging activity of honey can be evaluated using a deoxyribose degradation assay. This assay is based on quantification of the yellow complexes, which are the products of the condensation of 2-deoxyribose's degradation product with thiobarbituric acid (TBA) (Pavithra and Vadivukkarasi, 2015). Theoretically, antioxidants in honey and ginger can eliminate hydroxyl radicals, which are formed by the reaction between ascorbic acid and ferric ethylenediaminetetraacetic acid (FeEDTA). Hydroxyl radical is a very powerful reactive oxygen species that can induce severe damage to the adjacent biomolecule.

The antioxidant activity can be detected by measuring the intensity of the yellow colour formed using a spectrophotometer at 532 nm (Zhou et al., 2012).

Besides, the antioxidant activities possessed by honey can be evaluated by its superoxide anion scavenging activity. Superoxide radicals were produced in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide or in short PMS-NADH system by the oxidation of NADH. This activity was measured based on the reduction of nitro blue tetrazolium into a purple formazan by honey. The quantity of formazan formed was determined by measuring the absorbance at 560 nm (Sharma and Ajay, 2012).

Moreover, the antioxidant properties of honey can be tested from its ability to chelate transition metal ions. Hence, the ferrous-ferrozine method is used to evaluate the iron chelating activity of honey. The ferrozine-ferrous complex formation can be disturbed by antioxidants in honey, leading to the discolouration of the red magenta complex (Yusof et al., 2013). This decrease in colour intensity can be measured using a spectrophotometer at wavelength 562 nm (Chai et al., 2014).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Honey Samples

As shown in Table 3.1 and Appendix A, three honey samples were provided by Eco Bee Shop Sdn. Bhd., Johor. Honey samples with 10 % and 18 % ginger extract were prepared by adding the ginger extract into the base honey, which is amultifloral honey. The multifloral honey sample was collected from the rainforest in Malaysia where the wild bee lives in. All honey samples were sealed tightly in a glass bottle and kept in dark at room temperature to avoid crystallization and direct sunlight exposure so that the quality of the honey can be maintained (Fasasi, 2012).

Table 3.1: The details of honey samples.

Symbol	Product name	Bee species
ORI	Rainforest wild raw honey	*Apis cerana*
H10	Ginger Honey Bentong (10 % ginger)	*Apis cerana*
H18	Ginger Honey Bentong (18 % ginger)	*Apis cerana*

3.1.2 Bacterial Samples

Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* were used in the antibacterial assays of this study. The reference strain *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were provided by Faculty of Science of UTAR. All the bacteria samples were maintained on nutrient agar.

3.1.3 Chemicals and Media

All the used chemicals and media are recorded in Appendix B (Table A).

3.1.4 Equipment and Labware

All the used equipment and labware are recorded in Appendix B (Table B).

3.2 Methodology

3.2.1 Research Methodology

Figure 3.1 shows the overview of the experimental flow in this project. The project began with the sensory evaluation of the honey sample and then followed by the analysis of six different physicochemical characteristics. Next, the well diffusion assay was carried out to assess the antibacterial ability of the honey samples. Finally, four antioxidant assays were conducted.

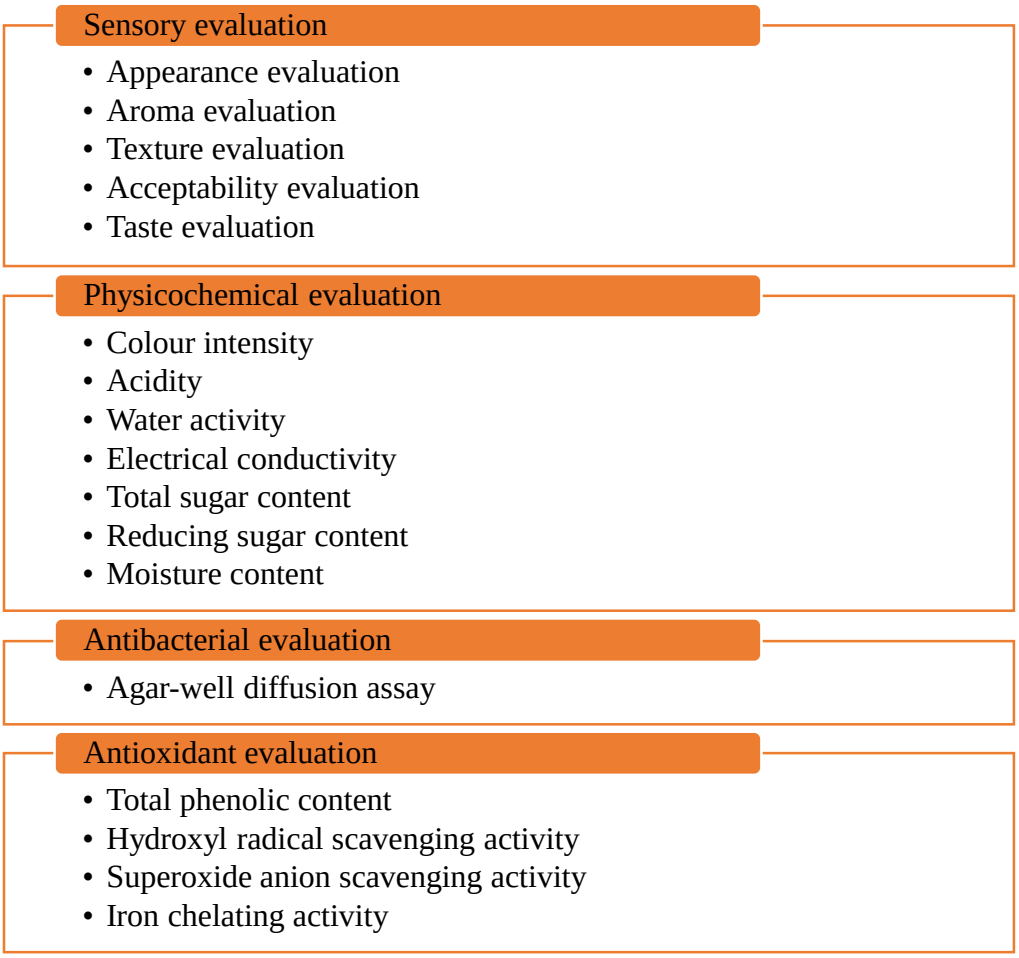


Figure 3.1: The overview of experimental design.

3.2.2 Preparation of Reagents

All the reagents used in the project are indicated in Appendix C.

3.2.3 Sensory Properties

The original honey sample, honey with 10 % addition of ginger, honey with 18 % addition of ginger and a control which is a sugar solution with 43 % fructose, 28 %

glucose and 2.0 % sucrose were evaluated by eight panelists for five attributes, which are appearance, aroma, taste, texture, and acceptability. The olfactory descriptors involved were woody, chemical, fresh, floral and fruity, warm, spoiled and vegetal while the selected taste descriptors were the same as the olfactory descriptors with additional astringency, refreshing, sweetness, sourness, saltiness, bitterness, persistence and after taste.

The panelists were selected, non-smoker food science and dietetics lecturers, students and staffs of Universiti Tunku Abdul Rahman due to their knowledge regarding sensory analysis and food product profiling. There were five women and three men, aged from 26 to 40 years. The panelists were required to evaluate the honey samples at least 2 hours after main meals. They were all briefed before the sensory analysis and exposed to the detailed description of each sensory attribute. The briefing guideline with attribute descriptions was shown in Appendix D.

The sensory evaluation was carried out in a sensory room. The assessment of each honey was done on a score sheet with yes or no questions and 5-point scales, from 1 (very weak or dislike very much) to 5 (very strong or like very much). The score sheet was shown in Appendix D. The control sugar solution was prepared freshly before the evaluation. Approximately 10 g of each sample were served to each assessor at room temperature by using capped, transparent glass vials. The honey samples were randomly coded and the panelists evaluated the honey samples at the

same time but in a different order to minimize their interaction. The panelists were informed to rinse their mouths using distilled water and smell the coffee bean between each sample to clean their palate and relax smell (Moumeh et al., 2020).

3.2.4. Colour Intensity

The colour intensity of honey samples was measured using the method of Beretta et al. (2005). A honey solution with a concentration of 50 % (w/v) was prepared by dissolving 1 g of honey sample into 2 mL of warm distilled water. The granules in the honey solution were filtered out by a 0.45 µm nylon syringe filter. The absorbance of the honey solution was read at 450 nm and 720 nm using FLUOstar® Omega microplate reader. The measurement was triplicated and the colour intensity was calculated using the formula listed below. The mean value was expressed as milli-absorbance unit (mAU).

$$\text{Colour Intensity} = Abs_{450} - Abs_{720}$$

3.2.5 Acidity

The pH of honey samples was measured using Eutech pH 2700 Meter. The pH meter was calibrated before the measurement. A honey solution with a concentration of 0.13 g/mL was prepared by adding 10 g of honey sample into 75 mL of distilled water. The measurement was triplicated and the mean pH value was calculated (Adenekan et al., 2010).

3.2.6 Water Activity

The water activity of honey samples was measured using Novasina Lab Swift portable water activity meter. The water activity meter was calibrated with salt standards provided before the measurement. Approximately 8 mL of honey sample was added into a container and then put into the measuring chamber. The measurement was carried out at room temperature and the reading was recorded in two minutes intervals. The measurement was triplicated and the mean value was calculated.

3.2.7 Electrical Conductivity

The electrical conductivity of honey samples was measured using OAKTON Multi-Parameter PCSTestrTM 35 (International Honey Commission, 2009). Approximately 6 g of honey sample was added into 30 mL of distilled water to form a honey solution with a concentration of 20 % (w/v). The measurement was performed in triplicated and the mean value was expressed as microSiemens per centimetre (µS/cm).

3.2.8 Total Sugar Content

The total sugar content of honey samples was measured using Atago® pocket refractometer. The calibration of the refractometer was performed by adding approximately 80 µL of distilled water on its measuring platform prior to

measurement. The distilled water was discarded after calibration and replaced with honey samples to measure its refractive index. The measurement was carried out thrice and the average value was expressed in percentage, % (g/100g) (Moniruzzaman et al., 2013).

3.2.9 Reducing Sugar Content

The reducing sugar content of honey samples was measured using 3, 5 dinitrosalicylic acid (DNSA) (Moniruzzaman et al., 2013). Approximately 1 g of honey sample was added into 10 mL of distilled water to form a honey solution with a concentration of 0.1 g/mL, then the honey solution was further diluted at 100-fold with distilled water, by dissolving 0.1 mL aliquot into 9.9 mL of distilled water to produce a final concentration of 0.001 g/mL. A series of glucose standard solutions were produced as shown in Appendix E (Table B). Subsequently, approximately 3 mL of distilled water, 3 mL of each honey sample, and 3 mL of each glucose standard solution were added with 3 mL of DNSA solution respectively. The mixture was incubated in a boiling water bath for 10 minutes and left to cold down before mixing with 1 mL of Rochelle salt. The absorbance of mixture was measured at 540 nm against a blank by using FLUOstar® Omega microplate reader. The measurement was triplicated. A standard curve of absorbance against concentration of glucose standard solution (200, 400, 600, 800, 1000 µg/mL) was plotted and the reducing sugar content was calculated using the formula listed below. The average value was expressed as percentage (%).

$$R = C \times 1/D$$

R = Total reducing sugar content in honey sample (%);

C = Concentration of glucose obtained from standard curve (µg/mL);

D = Density of honey sample (g/mL).

3.2.10 Moisture Content

The moisture content of honey samples was determined by its refractive index so the Atago® pocket refractometer was used to measure the moisture content of honey directly. The refractometer was calibrated with distilled water before use. The measurement was triplicated and moisture content was calculated by using the formula listed below. The average value was recorded as percentage (%) (AOAC, 1990).

Moisture Content (%) = 100 % - Total Sugar Content

3.2.11 Antibacterial Properties

The antibacterial ability of each honey sample was assessed using agar well diffusion assay modified by Mohapatra et al. (2011). Approximately 25 mL of Muller-Hinton agar was got ready on a sterile petri dish. After that, fresh bacteria culture *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923

were inoculated into a tube with 8 mL of sterile 0.85 % normal saline respectively. The turbidity of the bacterial suspension was regulated to 0.5 McFarland, at which its optical density reading is between 0.08 to 0.13 at wavelength 625 nm, equivalent to 1×10^8 CFU/mL (Andrews, 2009). The tip of a cotton swab was dipped into the bacterial suspension and pressed against the wall of the tube with firm pressure to remove the excess. Next, the bacteria were streaked over the entire surface of agar plate evenly. A sterile 6 mm diameter cork borer was used to create four wells on the agar for three agar plates and two wells on agar for one agar plate. Approximately 90 µL of each honey sample and sugar solution were filled into the four wells of three agar plates while 90 µL of distilled water that serves as negative control and 90 µL of ampicillin solution (10 µg/mL) that serves as positive control were filled into two wells of another agar plate. The agar plates were incubated at 37°C overnight and then the zone of inhibition's diameter was measured to the nearest centimeter (cm). The assay was triplicated and the average diameter was calculated.

3.2.12 Total Phenolic Content

The total phenolic content of each honey sample was evaluated via a modified Folin-Ciocalteu method (Khalil et al., 2012). Gallic acid standard solutions were prepared according to Table A in Appendix E. All 0.2 g/mL honey samples diluent were prepared by adding 1 g of honey sample into 5 mL of distilled water. Approximately 0.5 mL of gallic acid standard solution and diluted honey sample were mixed with 0.5 mL of Folin and Ciocalteu's phenol reagent and left for 3

minutes. Then, 0.5 mL of 10 % (w/v) sodium carbonate solution and distilled water were added into the mixtures to reach a final volume of 5 mL. Incubation of the mixtures was carried out in dark and at room temperature for 90 minutes. The blank was the mixture of the reagents with distilled water. The absorbance of each mixture was read at wavelength 725 nm against the blank using FLUOstar® Omega microplate reader. The assay was triplicated and a standard curve of absorbance against gallic acid standard solution concentration was plotted. The total phenolic content of honey samples were calculated using the formula shown below and the final value was expressed as milligram of gallic acid equivalent (GAE) per kilogram of honey.

$$T = C \times V/M$$

T = Total phenolic compounds in honey sample (mg GAE/kg);

C = Concentration of gallic acid derived from standard curve (mg/mL);

V = Volume of honey sample used (mL);

M = Mass of honey sample used (kg).

3.2.13 Hydroxyl Free Radical Scavenging Assay

The deoxyribose degradation assay proposed by Zhou et al. (2012) was used to test the effectiveness of honey samples to scavenge hydroxyl radicals. All the diluted honey samples with a concentration of 0.2 g/mL were prepared by putting 1 g of honey sample into 5 mL of distilled water. Then, the diluted honey sample was

added with 400 µL phosphate buffer at a concentration of 0.2 M and pH 7.4, 50 µL of 2-deoxyribose reagent with a concentration of 50 mM, 50 µL of 1 mM edetate disodium salt dihydrate reagent, 50 µL of 3.2 mM ferric citrate solution, 50 µL of 50 mM hydrogen peroxide solution and 50 µL of 1.8 mM ascorbic acid. After the 20 minutes incubation of the mixture at 50 °C, 250 µL 10 % trichloroacetic acid and 150 µL of 5 % thiobarbituric acid in sodium hydroxide solution were added into the mixture. The mixture was then incubated in a 95 °C water bath for 15 minutes. The absorbance of each sample was measured at 530 nm by using FLUOstar® Omega microplate reader. The assay was repeated thrice and the blank was prepared by replacing the honey sample with distilled water. The reduction of hydroxyl radical was represented in the percentage of inhibition of deoxyribose degradation according to the equation below:

$$\% \text{ inhibition} = (A_0 - A_t)/A_0 \times 100$$

A_0 = Absorbance of the blank

A_t = Absorbance of sample

3.2.14 Superoxide Anion Scavenging Activity

The superoxide anion scavenging activity of each honey sample was evaluated using a modified method proposed by Chai et al. (2014) and Amir et al. (2011). The honey samples were diluted to 0.2 g/mL by adding 1 g of honey sample into 5 mL of distilled water and the ginger honey samples were filtered by 0.45 um nylon

syringe filters. A mixture containing 0.8 mL of potassium phosphate buffer at a concentration of 100 mM and pH of 7.4, 0.1 mL of nitroblue tetrazolium at a concentration of 0.78 mM, 0.1 mL of beta-Nicotinamide adenine dinucleotide solution at a concentration of 2.34 mM, 0.05mL of phenazine methosulfate at a concentration of 0.06 mM and 0.1 mL of honey sample was prepared and incubated at 25°C in dark for 20 minutes. Then, the absorbance of each sample was measured at 560 nm with FLUOstar® Omega microplate reader. The assay was performed in triplicate. The superoxide anion scavenging activity of honey sample was calculated according to the following equation and the values were recorded in percentage of inhibition.

$$\% \text{ Inhibition} = 1 - (A_t / A_0) \times 100$$

A_t = Absorbance of sample

A_0= Absorbance of control

3.2.15 Iron Chelating Activity

The honey samples were evaluated for iron chelating activity by using the ferrous-ferrozine complex method of Chai et al. (2014). Ferrozine solution in a concentration of 0.25 mM and ferrous sulphate solution in a concentration of 0.1 mM were prepared as stated in Appendix C. The 0.2 g/mL dilution of each honey sample was made by putting 1 g of honey into 5 mL of distilled water. An aliquot of 0.2 mL of each honey sample was mixed with 0.4 mL of ferrozine solution and 0.2 mL of ferrous sulphate solution. The negative control was prepared by replacing

the honey samples with distilled water. The mixture was then incubated at room temperature for 10 minutes and the ginger honey samples were filtered with 0.45 um nylon syringe filters after incubation. The absorbance was measured against a blank at a wavelength of 562 nm with FLUOstar® Omega microplate reader. The assay was triplicated and the iron chelating activity in percentage was calculated according to the following formula.

$$\text{Chelating activity (\%)} = 1 - (As/Ac) \times 100$$

As = Absorbance of sample;

Ac = Absorbance of control.

3.2.16 Statistical Analysis

All the assays were performed in triplicates and the final results were expressed in mean with standard deviation. The mean value differences between honey samples were analyzed by one-way analysis of variance (ANOVA) using Microsoft Excel 2013. A statistically significant test result with two-tailed $p < 0.05$ was achieved.

CHAPTER 4

RESULTS

4.1 Sensory Properties

4.1.1 Olfactory Evaluation

Referring to the results in Table 4.1 and Figure 4.1, the sugar solution did not come with fresh, floral fruity and warm odour. In contrast, woody, fresh and warm were the top three distinguishable odour attributes from all the analyzed honey samples. All honey evaluated were found to have a similar percentage for most of the odour attributes as three of them got the same percentage for chemical (12.5 %), fresh (62.5 %) and vegetal (37.5 %) odour. The exceptions were the floral fruity odour that showed the lowest percentage of 12.5 % in the H10 honey and spoiled odour that showed the lowest value of 12.5 % in the ORI honey. The ORI honey was perceived for the odour of floral fruity (62.5 %) and warm (87.5 %). Furthermore, the percentage for the woody attribute in the honey varied from 62.5 % (ORI) to 100.0 % (H10) whereas H18 honey had an intermediate percentage of 87.5 %. Taken as a whole, the addition of ginger resulted in the rise of woody and spoiled intensity while decreasing the intensity of floral fruity and warm smell.

Table 4.1: Result of olfactory evaluation of honey samples.

Attribute	Average Score				Percentage (%)			
	ORI	H10	H18	SS	ORI	H10	H18	SS
Woody	0.625	1.000	0.875	0.625	62.5	100.0	87.5	62.5
Chemical	0.125	0.125	0.125	0.250	12.5	12.5	12.5	25.0
Fresh	0.625	0.625	0.625	0	62.5	62.5	62.5	0
Floral fruity	0.625	0.125	0.500	0	62.5	12.5	50.0	0
Warm	0.875	0.750	0.750	0	87.5	75.0	75.0	0
Spoiled	0.125	0.375	0.375	0.125	12.5	37.5	37.5	12.5
Vegetal	0.375	0.375	0.375	0.250	37.5	37.5	37.5	25.0

ORI indicated rainforest wild raw honey.

H10 indicated ginger honey bentong with 10 % ginger.

H18 indicated ginger honey bentong with 18 % ginger.

SS indicated sugar solution.

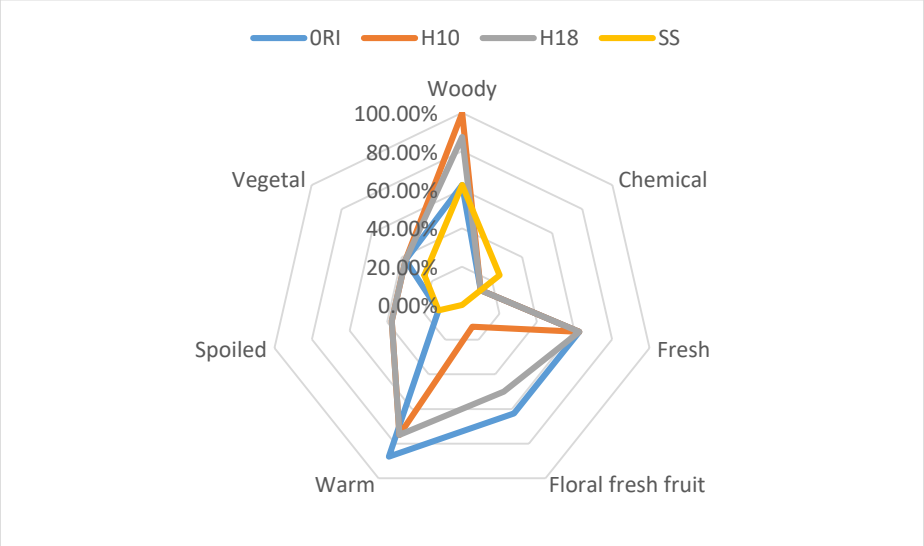


Figure 4.1: Radar chart of olfactory evaluation of honey samples.

4.1.2 Taste Evaluation

The sensory taste descriptors and results are presented in Table 4.2 and Figure 4.2. The sugar solution did not come with fresh, astringency and refreshing taste but these tastes were detected in all honey samples. The well-perceptible tastes in honey were woody, sweetness and warm. The H10 and H18 ginger honey were characterized by all of the taste attribute tested, while the chemical and spoiled attributes were not perceived in the base ORI honey. The highest level of woody (100.0 %), chemical (25.0 %), vegetal (50.0 %), sourness (42.5 %) and saltiness (45.0 %) attributes were found in H10 honey. Correspondingly, the ORI honey scored the best for sweetness (82.5%), floral fruity (62.5 %), warm (87.5 %), bitterness (42.5 %), persistence (77.5 %) and after taste (80.0 %) attribute. Similarly, the highest percentage of astringency was 37.5 % obtained in the H18 honey. Three honey samples attained the same percentage for the fresh and refreshing attributes with 62.5 % and 37.5 % respectively. As a whole, the addition of ginger to the base honey reduced its sweetness and gave the ORI honey extra flavours, which are the chemical and spoiled taste.

Table 4.2: Result of taste evaluation of honey samples.

Attribute	Average Score				Percentage (%)			
	ORI	**H10**	**H18**	**SS**	**ORI**	**H10**	**H18**	**SS**
Woody	0.500	1.000	0.750	0.500	50.0	100.0	75.0	50.0
Chemical	0	0.250	0.125	0.250	0	25.0	12.5	25.0
Fresh	0.625	0.625	0.625	0	62.5	62.5	62.5	0
Floral fruity	0.625	0.250	0.375	0.375	62.5	25.0	37.5	37.5
Astringency	0.250	0.250	0.375	0	25.0	25.0	37.5	0
Warm	0.875	0.750	0.625	0.250	87.5	75.0	62.5	25.0
Spoiled	0	0.125	0.125	0.250	0	12.5	12.5	25.0
Vegetal	0.125	0.500	0.375	0.250	12.5	50.0	37.5	25.0
Refreshing	0.375	0.375	0.375	0	37.5	37.5	37.5	0
Sweetness	4.125	2.750	2.250	2.000	82.5	55.0	45.0	40.0
Sourness	2.000	2.125	1.875	1.750	40.0	42.5	37.5	35.0
Saltiness	2.000	2.250	2.125	1.750	40.0	45.0	42.5	35.0
Bitterness	2.125	1.875	2.000	1.500	42.5	37.5	40.0	30.0
Persistence	3.875	3.750	3.500	1.625	77.5	75.0	70.0	32.5
After taste	4.000	3.750	3.375	1.625	80.0	75.0	67.5	32.5

ORI indicated rainforest wild raw honey.

H10 indicated ginger honey bentong with 10 % ginger.

H18 indicated ginger honey bentong with 18 % ginger.

SS indicated sugar solution.

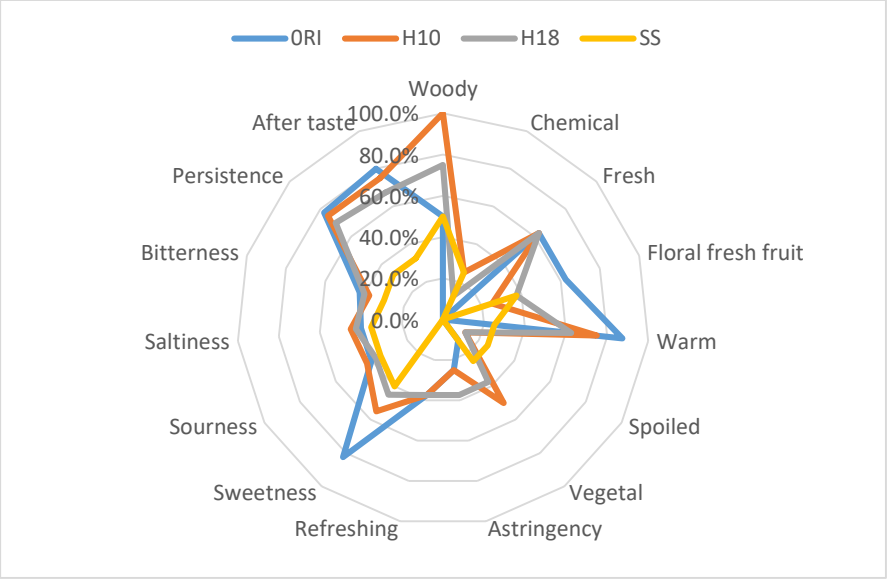


Figure 4.2: Radar chart of taste evaluation of honey samples.

4.1.3 Visual, Texture and Acceptability Evaluation

According to Table 4.3 and Figure 4.3, the colour intensity of all tested samples varied from 52.5 % to 62.5 %. The H18 honey scored the highest colour intensity of 62.5 % among three honey samples, followed by H10 honey with 60.0 % and ORI honey with 52.5 %. Next, the H10 had the greatest viscosity of 72.5 % and the H18 honey had the lowest viscosity of 67.5 %. The ORI honey with 70.0 % viscosity was in between them. For the adhesiveness, ORI honey showed the highest percentage of 70.0 %, the H18 honey showed the second-highest percentage of 57.5 % and the H10 honey showed the lowest percentage of 55.0 %. In general, ORI honey was the best accepted by the panelists (70.0 %), subsequently were H10 honey (55.0 %) and H18 honey (52.5 %). The sugar solution with 40.0 % marked the lowest acceptability among all samples.

Table 4.3: Result of visual, texture and acceptability evaluation of honey.

Attribute	Average Score				Percentage (%)			
	ORI	H10	H18	SS	ORI	H10	H18	SS
Colour intensity	2.625	3.000	3.125	3.125	52.5	60.0	62.5	62.5
Viscosity	3.500	4.250	3.375	1.125	70.0	85.0	67.5	22.5
Adhesiveness	3.500	3.625	2.875	1.625	70.0	72.5	57.5	32.5
Acceptability	4.500	2.750	2.625	2.000	90.0	55.0	52.5	40.0

ORI indicated rainforest wild raw honey.

H10 indicated ginger honey bentong with 10 % ginger.

H18 indicated ginger honey bentong with 18 % ginger.

SS indicated sugar solution.

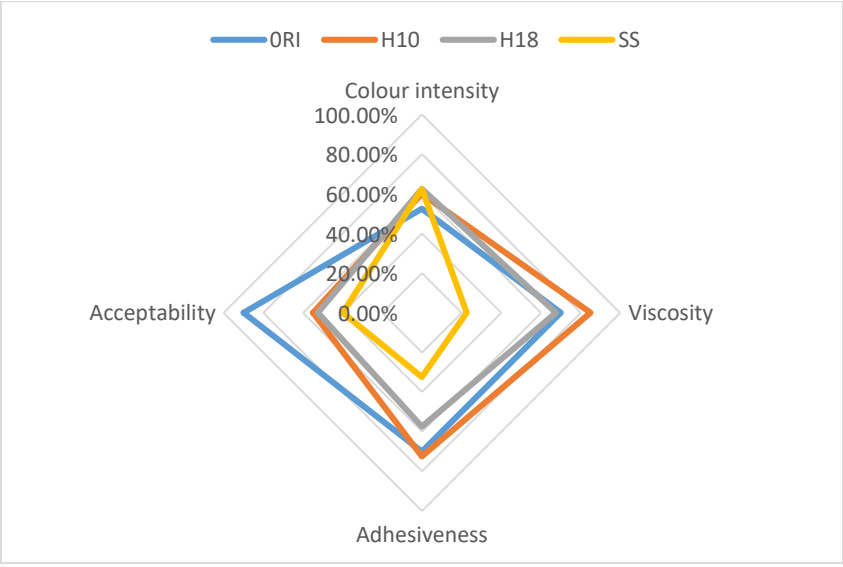


Figure 4.3: Radar chart of visual, texture and acceptability evaluation of honey.

4.2 Physicochemical Properties

Physicochemical properties are important indicators for the authenticity and quality of honey. As presented in Table 4.4, the highest colour intensity was exhibited by the H10 ginger honey with 304.50 mAU, followed by the H18 ginger honey with 268.00 mAU and base honey with 206.00 mAU. Thus, the H10 ginger honey is the darkest honey among these three samples. According to Table 4.4, all the tested honey samples were ranged from pH 4.53 - 4.88, indicating all honey samples were acidic and within the limit (pH 3.40 to 6.10) that indicates freshness. The pH of the base honey was 4.53, H10 ginger honey was 4.84 and H18 ginger honey was 4.88. Besides, the H18 ginger honey showed the highest water activity of 0.55, followed by the H10 ginger honey with a water activity of 0.54 and the water activity for ORI base honey was only 0.54, the lowest among the three samples. From Table 4.4, the electrical conductivity of all honey samples was between 290.00 µS/cm to 532.30 µS/cm so all of them achieved the parameter set by the Codex Alimentarius Commission (2001) which stated the electrical conductivity of honey should not be more than 800.00 µS/cm. The highest electrical conductivity belonged to H18 ginger honey (532.30 µS/cm), the lowest electrical conductivity belonged to ORI honey (290.00 µS/cm) and the H10 ginger honey (444.70 µS/cm) was in between. Overall, there were significant differences in colour intensity, pH and electrical conductivity between the base honey and ginger honey samples where the ginger honey samples were darker in colour, less acidic and showed a higher ability to conduct electricity than the base honey. However, there was no significant difference in water activity between the base honey and ginger honey samples.

Table 4.4: Colour intensity, pH, water activity and electrical conductivity of three tested honey samples.

Honey samples	Colour intensity (mAU)	pH	Water activity	Electrical conductivity (µS/cm)
ORI	206.00 ± 2.00[a]	4.53 ± 0.02[a]	0.54 ± 0.00	290.00 ± 3.61[a]
H10	304.50 ± 2.12[c]	4.84 ± 0.01[c]	0.54 ± 0.00[c]	444.70 ± 3.06[c]
H18	268.00 ± 2.83[b]	4.88 ± 0.01[b]	0.55 ± 0.00[b]	532.30 ± 3.21[b]

The assay was performed in triplicates and the average value was expressed in mean ± standard deviation.

a indicated significant difference between ORI and H10 with $p < 0.05$.

b indicated significant difference between ORI and H18 with $p < 0.05$.

c indicated significant difference between H10 and H18 with $p < 0.05$.

As displayed in Table 4.5, the total sugar content of ORI honey was 80.0 %, H10 honey was 81.3 % and H18 honey was 78.6 % while the reducing sugar content of ORI honey was 57.12 %, H10 honey was 59.64 % and H18 honey was 61.92 %. Thus, only the H18 honey fulfilled the international standard of the Codex Alimentarius Commission (2001) that limited the reducing sugar content of honey to more than 60.00 %. In this study, the moisture content of all tested honey samples was between 18.7 % and 21.4 %. The international honey quality regulation established by Codex Alimentarius Commission (2001) limited the moisture content of honey to 20.0 % or below. The H18 ginger honey was the only tested honey that exceed the limit with the moisture content of 21.4 % while both ORI base honey and H10 ginger honey were within the limit with the moisture

contents of 20.0 % and 18.7 % respectively. As summarized in Table 4.5, the reducing sugar content of both ginger honey samples was significantly higher than the base honey.

Table 4.5: Total sugar content, reducing sugar content and moisture content of three tested honey samples.

Honey samples	Total sugar content (%)	Reducing sugar content (%)	Moisture content (%)
ORI	80.0 ± 0.00	57.12 ± 0.57[a]	20.0 ± 0.00
H10	81.3 ± 0.00	59.64 ± 0.75[c]	18.7 ± 0.00
H18	78.6 ± 0.00	61.92 ± 0.61[b]	21.4 ± 0.00

The assay was performed in triplicates and the average value was expressed in mean ± standard deviation.

a indicated significant difference between ORI and H10 with $p < 0.05$.

b indicated significant difference between ORI and H18 with $p < 0.05$.

c indicated significant difference between H10 and H18 with $p < 0.05$.

4.3 Antibacterial Properties

The antibacterial properties of all honey samples were assessed by the agar well diffusion method and the results obtained were recorded in Table 4.6. The sugar solution did not induce any inhibitory effect on the growth of both tested bacteria. On the other hand, all tested honey samples were able to limit the growth of both

Escherichia coli and *Staphylococcus aureus*. H18 honey sample exerted the strongest inhibitory effect against both *E. coli* and *S. aureus* by producing a zone of inhibition with a diameter of 1.05 cm and 1.03 cm respectively. H10 honey sample displayed zone of inhibition in diameter of 0.98 cm against *E. coli* and 0.95 cm against *S. aureus*. Meanwhile, ORI honey was the weakest antibacterial agent as it only created zone of inhibition with 0.90 cm diameter against both *E. coli* and *S. aureus.* Generally, the results showed the absence of significant differences between the antibacterial activities of three honey samples although both of the ginger honey samples exhibited slightly higher inhibitory effects against both *E. coli* and *S. aureus* than the base honey.

Table 4.6: Antibacterial activity of tested honey against reference strains.

Honey samples	**Diameter of zone of inhibition (cm)**	
	***Escherichia coli* ATCC 25922**	***Staphylococcus aureus* ATCC 25923**
Sugar solution	NIL	NIL
ORI	0.90 ± 0.10	0.90 ± 0.09
H10	0.98 ± 0.03	0.95 ± 0.10
H18	1.05 ± 0.05	1.03 ± 0.06

The assay was performed in triplicates and the average value was expressed in mean ± standard deviation.

NIL = No zone of inhibition

4.4 Antioxidant Properties

The results for the four antioxidant assays carried out in this study were tabulated in Table 4.7. The lowest value of 20.19 mg GAE/kg for the total phenolic content was determined in the ORI honey, then the total phenolic content rise further in H10 and H18 ginger honey with 37.08 mg GAE/kg and 45.95 mg GAE/kg correspondingly. Furthermore, the hydroxyl free radical scavenging activity of three honey samples ranged from 69.58 % to 71.75 %. The highest percentage of 71.75 % was achieved by H18 honey, the lowest percentage of 69.58 % was achieved by ORI honey whereas the percentage of 71.01 % achieved by H10 honey was in between. Besides, the superoxide anion scavenging activity for the ORI honey was the lowest. Only 56.27 % activity inhibition was determined in ORI while H10 and H18 honey presented higher values. H10 honey showed 80.74 % inhibition and H18 honey showed 85.55 % inhibition. Similarly, for the iron chelating assay, H18 exhibited the highest activity of 21.28 %, followed by H10 with 13.32 % and ORI honey with 1.64 %. On the whole, the antioxidant activity of tested honey samples increased in the order of ORI honey, H10 honey and H18 honey. On the other hand, the differences in the total phenolic content, superoxide anion scavenging activity and iron chelating activity between ginger honey samples and base honey were statistically significant.

Table 4.7: Results of antioxidant assays of four tested honey samples.

Honey samples	Total phenolic content (mg GAE/kg)	Hydroxyl free radical scavenging activity (%)	Superoxide anion scavenging activity (%)	Iron chelating activity (%)
ORI	20.19 ± 0.00^{a}	69.58 ± 0.48	56.27 ± 0.38^{a}	1.64 ± 0.51^{a}
H10	37.08 ± 0.00^{c}	71.01 ± 0.78^{b}	80.74 ± 2.90^{c}	13.32 ± 5.08
H18	45.95 ± 0.00^{b}	71.75 ± 0.54	85.55 ± 0.66^{b}	21.28 ± 1.99^{b}

The assay was performed in triplicates and the average value was expressed in mean ± standard deviation.

a indicated significant difference between ORI and H10 with $p < 0.05$.

b indicated significant difference between ORI and H18 with $p < 0.05$.

c indicated significant difference between H10 and H18 with $p < 0.05$.

CHAPTER 5

DISCUSSION

5.1 Sensory Properties

In this study, the taste and odour of honey with ginger generally differed from the control honey. The addition of ginger resulted in the increase of taste and odour intensity of woody and spoiled as well as the decline of taste and odour intensity of floral fruity. The ginger addition also gave additional chemical and vegetal taste to the base honey but weakened the honey's sweet taste. This can be due to the pungent and spicy properties as well as the herbal nature of ginger (Technical University of Munich, 2018). By comparing the findings in this study with other research, there are very limited information on the sensory properties of multifloral honey and most of the research works only evaluated the basic tastes like sweetness and sourness but did not focus on the other taste and odour attributes. Only Araujo et al. (2020) described multifloral honey from Venezuela came with a sweet but slightly sour taste and floral, acid fruit olfactory attributes, which was similar to the results of this study.

In contrast, all honey analyzed had similar scores for colour intensity, viscosity and adhesiveness. However, the acceptability of honey in the present study decreased

with the addition of ginger from 90.0 % acceptability for ORI, 55.0 % for H10 and 52.5 % for H18. This result contrast with the result of a study using multifloral control honey and honey–ginger products as samples (Aleksandra et al., 2017). The previous study obtained 48.2 % for the acceptability of multifloral honey, which was much lower than the value in the current study. In the same study, the authors pointed out that the addition of 1 % and 2 % ginger extract into the control honey increased the acceptability of honey to 65.6 % and 85.0 % respectively, which were higher than the ginger honey samples in this study. This difference could be explained by the higher amount of ginger (10 % and 18 %) added in the present study that high pointed the woody herbal flavour but attenuated the honey's original flavour (Technical University of Munich, 2018). On the other hand, ginger is believed to increase the mineral content of the honey mixture (Omoya and Akharaiyi, 2012). The honey taste can be influenced by the mineral content as the higher the mineral content, the stronger the metallic flavour of the honey which may not be preferred by the consumers (González et al., 2005).

5.2 Physicochemical Properties

5.2.1 Colour Intensity

The colour of honey samples with ginger varied between 268.00 mAU and 304.50 mAU, which were significantly darker than the base honey (206.00 mAU). Especially, the H10 honey colour gave the highest values (304.50 mAU). The colour intensity of ORI honey was also compared with the value reported by

Moniruzzaman et al. (2013) for multifloral honey (544.30 mAU) but the previously reported value was much higher than the values in this study. Colour intensity of honey is mainly influenced by its ash content, storage time and the presence of antioxidant pigments. Therefore, honey with darker colour contains more antioxidant pigments like carotenoids and flavonoids which contributed to more antioxidant activity than honey with a lighter colour (Ahmed et al., 2016). Darker colour observed in ginger honey samples than the ORI honey in this study can be due to the transfer of additional antioxidant components and minerals from ginger, increasing the ash content and antioxidant pigments in the mixture (Omoya and Akharaiyi, 2012). Additionally, there is no research reported on the colour intensity of the honey-ginger mixture.

5.2.2 Acidity

According to Bogdanov et al. (2008), the acidity of honey depends on the sugar fermentation that forms organic acid. The organic acid is responsible for the honey taste and its resistance to bacterial spoilage. The pH value of analyzed honey samples ranged between 4.53 and 4.88. They were all acidic and within the reference range of the National Honey Board (2003), which is from 3.4 to 6.1. The pH values of the ORI honey (4.53) in this study were higher than the value of 3.88 reported for multifloral honey from China (Wu et al., 2020). These results were also compared with the values published Araujo et al. (2020) for multifloral honey from Venezuela, the pH values in the current study were higher than the range of

3.60 and 4.10. The ORI honey samples were more acidic than the ginger honey samples, which showed that the addition of ginger affected the pH of the honey. Nevertheless, the pH value of ginger honey was increasing with its ginger content. This could be caused by the nature of ginger as an alkaline food that increased the pH of ginger honey samples (Purdie, 2019).

5.2.3 Water Activity

As shown in Table 4.4, the water activity of examined honey samples was between the range of 0.54 to 0.55. There was no significant difference in the water activity found between the ginger honey with the base honey. The results in the current study were in agreement with those found by Nebojša et al. (2020) for multifloral honey from Serbia which ranged from 0.53 to 0.63 but much higher than the values for other multifloral honey reported by Straumite et al (2011), at which the corresponding values were 0.19 to 0.32. Water activity is the total free water in honey and it is an important factor related to the quality of honey (Olaitan et al., 2007). Bacteria, molds and yeast need water activity as low as 0.90, 0.70 and 0.80 correspondingly for their growth (Jimenez et al., 2016). The water activity in all the tested honey samples was lower than the growth-required limit for bacteria, molds and yeast so all of the honey samples were resistant to the fermentation by those microbes. Referring to the findings of Chen (2019), water activity established a linear relationship with moisture content in honey, at which honey with higher water activity showed a higher moisture content. This relationship can be clearly

noted in this study as the H18 with the highest water activity also exhibited the highest moisture content. To date, there is very limited literature investigation on the water activity of honey- ginger mixture.

5.2.4 Electrical Conductivity

The electrical conductivity of all honey samples in this study fell in the range between 290.00 µS/cm to 532.30 µS/cm. The obtained mean values were all lower than the maximum 800.00 µS/cm recommended by Codex Alimentarius Commission (2001), designating the absence of heavy metals in the honey (Fredes and Montenegro, 2006). The electrical conductivity of the tested ginger honey sample was comparable to the values recorded in the study of Beykaya (2021), in which the electrical conductivity of the honey-ginger mixture was 550.00 µS/cm. However, the value for ORI honey in this study was lower than the findings in multifloral honey from Venezuela, which were between 370.00 to 470.00 µS/cm. Previous studies revealed that the electrical conductivity is closely related to its acidity as well as mineral content in the honey (Yücel and Sultanog, 2013). Likewise, the electrical conductivity changed when the amount of pollen in the honey decreased (Kaskoniene et al., 2010). The values of electrical conductivity for H10 and H18 honey were significantly higher in comparison with the ORI honey, indicating the higher mineral content and acid content in the mixture because the rich mineral content of the ginger added into the honey (Omoya and Akharaiyi, 2012).

5.2.5 Sugar Content

The total sugar content is closely related to the osmotic pressure and acidity of honey, at which the acidity and osmotic pressure increase proportionally with the increasing total sugar content. High osmotic pressure is useful in microbial growth inhibition of honey (Snowdon and Cliver, 1996). The total sugar content of all honey samples in the current study ranged between 78.60 % and 81.30 %. These results of H10 and H18 are compared to the results of Okeola et al. (2015), who obtained closely related findings of total sugar ranging from 68.20 % to 76.10 % for honey-ginger mixture. Overall, the previously reported values were lower than the values in this study. On the other hand, the results in the current study were higher than those found for other Malaysian honey with total sugar content ranging from 63.33 % to 68.40 % (Moniruzzaman et al., 2013). Theoretically, high sugar content can lead to more sugar fermentation that generates acidic products which further reduce the pH of the honey (Moniruzzaman et al., 2013). Also, another study by Can et al. (2015) observed a negative correlation between the moisture content of honey and its total sugar content. However, both types of correlation stated were not found in this study.

Honey consists of monosaccharides and oligosaccharides. In the current study, the reducing sugars were determined to be 57.12 %, 59.64 % and 67. 92 % in ORI, H10 and H18 respectively. The results proved that the dominant sugars in honey were fructose and glucose. According to Beykaya (2021), the proportion of glucose and

fructose present in honey were approximately equal to each other, at which glucose was observed to be between 26.0 % to 30.9 % while fructose was observed to be between 31.5 % to 39.3 %. The results of ginger honey obtained in the current study were similar to the previously reported values of other ginger honey samples by Okeola et al. (2015), for which the corresponding value was 65.00 %. When compared with the results of other Malaysian honey in the study of Moniruzzaman et al. (2013) that ranged between 61.17 % to 63.89 %, the values of honey samples in this study were slightly lower. As H18 honey was the only sample that showed a value of more than 60.00 % for reducing sugar content, it was the only honey among the three samples that met the international standard launched by Codex Alimentarius Commission (2001). Generally, the tested ginger honey samples showed higher reducing sugar content than the base honey and the reducing sugar content was found to have a going uptrend with the rising ginger content. This could be due to the donation of glucose and fructose from ginger to the honey since reducing sugars built up 2.2 % of ginger carbohydrates (Lee et al., 2014).

5.2.7 Moisture Content

In the present study, the moisture content of the examined honey samples was 20.00 % for ORI, 18.70 % for H10 and 21.40 % for H18. The data obtained in this study was consistent with the results of other Malaysian honey investigated by Khalil et al. (2010), which fell in the range of 12.79 % to 22.32 %. The values of ginger honey were comparable to the reported value of other ginger honey (22.13 %)

by Beykaya (2021) whereas the value of ORI honey was higher than the reported values of other *A. cerana* honey (15.70 %-18.60 %) by Akwal et al. (2020). Generally, only H10 fell within the maximum acceptable content for honey established by the International Honey Commission (< 20 %) while ORI and H18 were higher than the limit. The honey's moisture content is a limiting factor for its quality, shelf life and resistance against fermentation. High moisture content could lead to a high probability of osmotolerant yeasts-induced honey fermentation during storage (Khalil et al., 2012). Honey fermentation may give spoiled and bitter tastes to the honey (Imtara et al., 2018). According to Chen (2019), water activity showed a linear relationship with moisture content and this relationship was also observed in this study as H18 scored the highest for both water activity and moisture content. The low moisture content in H10 suggested that H10 was in good quality and proper storage condition. Meanwhile, observed high values of moisture content in ORI and H18 could be an indication for the extraction of unripe honey or the humid storage condition (Sahney and Kumar, 2017).

5.3 Antibacterial Properties

The antibacterial properties of honey were evaluated by using the agar well diffusion method. The Gram-negative bacteria and Gram-positive bacteria included in this evaluation were *Escherichia coli* and *Staphylococcus aureus*, the most common causative bacteria of bacteremia and healthcare-associated infections (Poolman and Anderson, 2018).

As shown in Table 4.6, the sugar solution that acted as the negative control did not show any inhibitory effects on the test bacteria while all the honey samples managed to inhibit both *E. coli* and *S. aureus*. This observation suggested the antibacterial effect of honey is not only depending on the sugar content in the honey but also counting on other bioactive components (Almasaudi, 2021). The inhibitory potency of all honey samples on the test bacteria was similar with non-significant higher inhibition was observed for ginger honey samples. Therefore, it could be said that the addition of ginger had no significant effect on the antibacterial activity of base honey although the combination of both honey and ginger was assumed to have an enhanced antibacterial ability because both of them are antimicrobial agents. This scenario could be explained by the probable antagonist reaction that occurs between the phytochemicals from honey and ginger (Aleksandra et al., 2017).

For *E. coli*, zone of inhibition of 0.90 cm formed by ORI honey, 0.95 cm by H10 and 1.03 cm by H18, were all lower than the previously reported values by Omoya and Akharaiyi (2011), which recorded 2.00 cm for pure honey and range of 2.80 cm-3.20 cm for honey-ginger mixture. For *S. aureus*, the diameter of zone of inhibition obtained in this study that ranged from 0.90 cm to 1.05 cm was also lower than the results recorded in the study of Omoya and Akharaiyi (2011), with 1.40 cm for pure honey and range of 2.60 cm-3.00 cm for honey-ginger mixture.

According to the study of Cooper et al. (1999), *S. aureus* is a bacteria species come with a higher tolerance in a high osmolarity environment and its high tolerance was also observed in the current study as all the tested honey samples formed larger zones of inhibition in *E. coli* and the lower inhibitory effect against *S. aureus.*

5.4 Antioxidant Properties

The total phenolic content of tested honey bee honey samples ranged from 20.19 mg GAE/kg to 45.95 mg GAE/kg. The value of ORI (20.19 mg GAE/kg) in this study was lower than the value obtained for multifloral honey (43 mg GAE/kg) in the study of Aleksandra et al. (2017). However, the current values were higher than the result reported by Sajak et al. (2019) for the honey-ginger mixture, which was 5 mg GAE/kg. In overall, the ginger honey samples contained significantly higher total phenolic content than the base honey. Since the phenolic compounds are used by plants to protect themselves from environmental stress and ginger is rich in phenolic compounds, it can be assumed that the rise in total phenolic content was due to the transfer of bioactive compounds from ginger to honey (Graf et al., 2010; Ali et al., 2018). According to Khalil et al. (2012), a positive correlation was found between colour intensity and the total phenolic content of honey in which the darker the honey colour, the higher its phenolic content. This finding is closely agreed by the results of this study because H18 honey with the highest total phenolic content showed higher colour intensity than H10 honey while H10 showed higher colour intensity than the base honey with the lowest total phenolic content.

Hydroxyl radical is the most reactive free radical because it has a high reduction potential toward every molecule in living organisms such as lipids, polypeptides, proteins, and DNA, forming the hydroxycyclohexadienyl radical (Nagai et al., 2012). Based on the results displayed in Table 4.7, the hydroxyl free radical scavenging activity in tested honey samples were between 69.58 % and 71.75 %. Significant differences were not witnessed among the samples although the trend of the scavenging activity increased with the increasing concentration of ginger in honey presented. As compared with the reported values ranging from 62.4 % -71.2 % in the study by Nagai et al (2012) using multifloral honey, the analyzed honey in this study showed a comparable result. Additionally, similar to this study, an increasing trend of hydroxyl free radical scavenging activity with the rising plant product concentration in honey was found in the study by Jasna (2014) that analyzed honey added with prune, another plant product that rich with antioxidant components. The non-significant increase of hydroxyl free radical scavenging activity in ginger honey can be due to the extra antioxidants that the ginger supplied to the base honey but the enhancing effect of ginger was restricted by the phytochemicals in honey (Aleksandra et al., 2017).

Superoxide anion is a free radical formed in the mitochondrial electron transport system, leading to the formation of other reactive oxygen species (Lee et al., 2004). The detrimental effect of the superoxide anion in living system is reduced by an

enzyme called superoxide dismutase (SOD) and honey was found to show similar activities as SOD (Hegazi and Abd El-Hady, 2009). The percentage inhibition of superoxide radical of all tested honey samples in the current study ranged from 56.27 % to 85.55 %. Based on the percentage inhibition of only ginger extract investigated by Yesiloglu et al. (2013), the values ranged from 28.6 % to 30.6 %. The samples used in this study showed much higher percentage inhibition compared to the previous study. Another study by Nagai et al. (2012) stated that the superoxide anion scavenging activity by multifloral honey was ranged between 12.4 - 66.6 %, which were lower than the values obtained in this study. Overall, both ginger honeys exhibited much stronger superoxide anion scavenging activity than ORI honey. The same trend was also reported in a similar study by Jasna et al. (2014) that examined the honey-prune products. In the prior study, the honey-prune products demonstrated a higher superoxide anion scavenging activity than honey without prunes. Additionally, the positive correlation between the total phenolic contents and superoxide anion radical scavenging activity reported in the study by Jasna (2014) was observed in this study too. Increased percentage inhibition of honey-ginger products could be explained by the fact that the combination of bioactive components from ginger and honey increased the antioxidant ability of the mixture so the mixture converted superoxide into hydrogen peroxide and oxygen more effectively (Sani et al., 2014).

Based on the previous studies, the iron chelating activity of multifloral honey was below 10.00 % and the iron chelating activity of ginger extracts were ranged from

27.00 % to 36.00 % (Bellik and Selles, 2017; Tohma et al., 2017). In the present study, the iron chelating activity for all the test honey samples was in between the range of 1.64 % to 21.28 %, which is higher than the previous data for multifloral honey but lower than the data for ginger extracts. Despite both honey and ginger showing the capacity in chelating metal ions, multifloral honey showed lower iron-chelating activity than ginger (Aleksandra et al., 2017). Moreover, ginger is well known as a natural source of metal chelators that could be used in the prevention of neurodegenerative diseases so the addition of ginger into honey contributed to the synergistic effect on its iron chelation (Sani et al., 2014; Tohma et al., 2017).

Generally, ginger honey in this study showed significantly higher iron chelating activity than the base honey and the iron chelating activity of honey was proportional to the ginger concentration in the honey as the H18 honey with the greatest added ginger concentration showed the greatest ability to interfere with the formation of ferrozine-ferrous complexes.

5.5 Future Studies

Adding spices to a base product is a popular method to enrich the nutritional value and the sensory quality of food. Multifloral honey produced by *Apis cerana* bee in Malaysia is one of the common honey types available in the local market whereas Bentong ginger is the most famous variety of *Zingiber officinale* in Malaysia. A novel product that merged the positive features of both multifloral honey and the

Bentong ginger should be able to broaden the benefit of honey products available in the current market. However, the scientific information on their combination is very limited. For this reason, future studies should be carried out to further identify the optimal Bentong ginger extract concentration added into the honey in order to get the best sensory, physicochemical, antioxidant and antibacterial properties of the ginger-honey mixture.

A wider range of ginger extract concentration can be added to the base honey to determine the minimal inhibitory concentration (MIC) so the minimal added ginger concentration to produce a positive effect on the antimicrobial properties of ginger-honey mixtures can be ascertained (Aleksandra et al., 2017). Likewise, a more advanced method like gas chromatography-mass spectrometry (GC-MS) analytical methods, e-nose and e-tongue can be applied to give a higher quality evaluation for the sensory attributes of ginger-honey mixtures (Heredia et al., 2013). On the other hand, the sugar profile in ginger-honey mixtures can be compared more accurately by using a high-performance liquid chromatograph equipped with a differential refractive index detector (Nazeh et al., 2015).

CHAPTER 6

CONCLUSION

In summary, the addition of ginger influenced the sensory properties, physicochemical characteristics and antioxidant activity of the base honey. The honey-ginger samples showed taste, aroma and texture differences with base honey. Pure honey was better accepted by consumers than ginger honey. Besides, honey-ginger mixtures were found to have more antioxidant effects than the pure honey due to the considerable amount of polyphenolics originating from honey and added ginger. In addition, the sensory properties, physicochemical characteristics and antioxidant activity were affected by the concentration of ginger added. The antioxidant effect of honey increased with the rising ginger concentration added into the honey. Therefore, this study proved that honey-ginger product is a good source of antioxidants. However, the addition of ginger had no significant effect on the antibacterial activity of honey. Generally, the tested honey samples met the standards set up by Codex Alimentarius Commission and International Honey Commission, excluding the electrical conductivity and moisture content.

REFERENCES

Abramovic, H., Jamnik, M., Burkan, L. and Kač, M., 2008. Water activity and water content in Slovenian honeys. *Food Control*, 19, pp. 1086–1090.

Abselami, A. et al., 2018. Physicochemical properties of some honeys produced from different flora of Eastern Morocco. *Journal of Materials and Environmental Science*, 9 (3), pp. 879 – 886.

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.

Ajibola, A., 2015. Physico-chemical and physiological values of honey and its importance as a functional food. *International Journal of Food and Nutritional Science*, 2(2), pp. 180-188.

Akwal, P., Meena, T., Kiran, R. and Sunita, Devi., 2020. Quality analysis of *Apis cerana* and *Apis mellifera* honey from Himachal Pradesh, India. *Journal of Entomology and Zoology Studies*, 8(6), pp. 46-54.

Alagesh, T.N., 2019, *Bentong ginger farmers to get new site* [Online]. Available at: https://www.nst.com.my/news/nation/2019/09/524080/bentong-ginger-farmers-get-new-site [Accessed 31 March 2022].

Aleksandra, W., Joanna, N. and Piotr, S., 2017. Influence of the addition of selected spices on sensory quality and biological activity of honey. *Journal of Food Quality*, 2017, pp. 1-7.

Al-Farsi, M., Al-Amri, A., Al-Hadhrami, A. and Al-Belushi, S., 2018. Color, flavonoids, phenolics and antioxidants of Omani honey. *Heliyon*, 4(10), pp. 114.

Ali, A. A., El-Nour, M. E. and Yagi, S. M., 2018. Total phenolic and flavonoid contents and antioxidant activity of ginger (*Zingiber officinale* Rosc.) rhizome, callus and callus treated with some elicitors. *Journal of Genetic Engineering and Biotechnology*, 16 (2), pp. 677-682.

Al-Mamary, M., Al-Meeri, A. and Al-Habori, M., 2002. Antioxidant activities and total phenolics of different types of honey. *Nutrition Research*, 22 (9), pp. 1041–1047.

Almasaudi, S., 2021. The antibacterial activities of honey. *Saudi Journal of Biological Sciences*, 28 (4), pp. 2188-2196.

Al-Waili, N. S., 2003. Effects of daily consumption of honey solution on hematological indices and blood levels of minerals and enzymes in normal individuals. *Journal of Medicinal Food*, 6 (2), pp. 135–140.

Alzoreky, N.S. and Nakahara, K., 2003. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *International Journal of Food Microbiology*, 80 (3), pp. 223-230.

Andrews, J.M., 2009. BSAC standardized disc susceptibility testing method (version 8). *Journal of Antimicrobial Chemotherapy*, 64(3), pp. 454-489.

Anh, N.H. et al., 2020. Ginger on human health: A comprehensive systematic review of 109 randomized controlled trials. *Nutrients*. 12(1), p.157.

Araujo, D. et al., 2020. Sensory profile and physico-chemical properties of artisanal honey from Zulia. Venezuela. *Foods*, 9(3), pp. 339 -350.

Aryal, S., 2018, Mueller Hinton Agar (MHA) – *Composition, Principle, Uses and Preparation* [Online]. Available at: https://microbiologyinfo.com/muellerhinton-agar-mha-composition-principle-uses-and-preparation/ [Accessed 20 February 2022].

Bakour, M. et al, 2022. Bee bread as a promising source of bioactive molecules and functional properties: An up-to-date review. *Antibiotics*, 11, pp. 203-241.

Baloš, M.Ž. et al., 2018. Electrical conductivity and acidity of honey. *Arhiv Veterinarske Medicine*, 11(1), pp. 91-101.

Balouiri, M., Sadiki, M. and Ibnsouda, S. K., 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), pp. 71–79.

Belay, A. et al., 2017. Sugar profile and physicochemical properties of Ethiopian monofloral honey. *International Journal of Food Properties*, 20(11), pp. 28552866.

Bellik, Y. and Selles, S. M. A., 2017. In vitro synergistic antioxidant activity of honey-*Mentha spicata* combination. *Journal of Food Measurement and Characterization*, 11(1), pp. 111-118.

Beretta, G., Granata, P., Ferrero, M., Orioli, M. and Facino, R.M., 2005. Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Analytica Chimica Acta*, 533(2), pp. 185-191.

Beristain-Bauza, S. D. C. et al., 2019. Antimicrobial activity of ginger (*Zingiber Officinale*) and its application in food products. *Food Reviews International*, 35(5), pp. 407-426.

Bertoncelj, J., Doberšek, U., Jamnik, M. and Golob, T., 2007. Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. *Food Chemistry*, 105 (2), pp. 822-828.

Bioquochem, 2019, *Folin Ciocalteau Phenolic Content Quantification Assay Kit* [Online]. Available at: https://filgen.jp/Product/Bioscience4/BioQuoChem/KB03-006%20Folin% 20Ciocalteau%20Book.pdf [Accessed 15 March 2022].

Blamire, J., 2000, *Effect of pH on growth rate* [Online]. Available at: http://www.brooklyn.cuny.edu/bc/ahp/CellBio/Growth/MGpH.html [Accessed 3 February 2022].

Bogdanov, S. et al., 1999. Honey quality and international regulatory standards: review by the International Honey Commission. *Bee World*, 80(2), pp. 61-69.

Bogdanov, S., Jurendic, T., Sieber, R. and Gallmann, P., 2008. Honey for nutrition and health: a review. *The Journal of the American College of Nutrition*, 27, pp. 677-689.

Brudzynski, K., Abubaker, K. and Wang, T., 2012. Powerful bacterial killing by buckwheat honeys is concentration-dependent, involves complete DNA degradation and requires hydrogen peroxide. *Frontiers in Microbiology*, 3(242), pp. 1-9.

Buba, F., Gidado, A. and Shugaba, A., 2013. Analysis of biochemical composition of honey samples from North-East Nigeria. *Biochemistry and Analytical Biochemistry*, 2, p. 139.

Burt, S., 2004. Essential oils: Their antibacterial properties and potential applications in foods—A review. *International Journal of Food Microbiology*, 94, pp. 223-253.

Can et al., 2015. An investigation of Turkish honeys: their physic-chemical properties, antioxidant capacities and phenolic profiles. *Food Chemistry*, 180, pp. 133-141.

Chai, T.T., Mohan, M., Ong, H.C. and Wong, F.C., 2014. Antioxidant, iron chelating and anti-glucosidase activities of *Typha domingensis* Pers (Typhaceae). *Tropical Journal of Pharmaceutical Research*, 13 (1), pp. 67 – 72.

Chang, C.C., Yang, M.H., Wen, H.M. and Chern, J.C., 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10, pp. 178–182.

Chen, C.H., 2019. Relationship between water activity and moisture content in floral honey. *Foods*, 8, pp. 30-48.

Codex Alimentarius Commission, 2001. Revised codex standard for honey. *Codex standard*, 12(1981), pp. 1-8.

Cooper, R.A., Molan, P.C. and Harding, K.G., 1999. Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. *Journal of the Royal Society of Medicine*, 92(6), pp. 283-285.

Delores, C.S., Christie, C. and Misra, R., 2004. Nutrition and well-being A to Z. *Turkish Journal of Veterinary and Animal Sciences*, 1 (7), pp. 20-23.

Dobre, I., Georgescu, L. A., Alexe, P., Escuredo, O. and Seijo, M. C., 2012. Rheological behavior of different honey types from Romania. *Food Research International*, 49(1), pp. 126–132.

El-Metwally, A.A.E., 2015. *Factors affecting the physical and chemical characteristics of Egyptian Beehoney*. PhD Thesis, Cairo University, Egypt.

El-Sharaky, A.S., Newairy, A.A., Kamel, M.A. and Eweda, S.M., 2009. Protective effect of ginger extract against bromobenzene-induced hepatotoxicity in male rats. *Food and Chemical Toxicology,* 47, pp. 1584–1590.

El-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.279–287.

Escuredo, O., Dobre, I., Fernández-González, M. and Seijo, M. C. (2014). Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. *Food Chemistry*, 149, pp. 84–90.

Escuredo, O., Míguez, M., Fernández-González, M. and Seijo, M. C. (2013). Nutritional value and antioxidant activity of honeys produced in a European Atlantic area. *Food Chemistry*, 138(2–3), pp. 851–856.

Estevinho, L. et al., 2008. Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food and Chemical Toxicolology*, 46, pp. 3774 – 3779.

Eteraf-Oskouei, T. and Najafi, M., 2013. Traditional and modern uses of natural honey in human diseases: a review. *Iranian Journal of Basic Medical Sciences*, 16(6), pp. 731-742.

Ewnetu, Y., Lemma, W. and Birhane, N., 2014, *Synergetic antimicrobial effects of mixtures of ethiopian honeys and ginger powder extracts on standard and resistant clinical bacteria isolates*. [Online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3977104/ [Accessed 11 October 2021].

Fasasi, K.A., 2012. Physicochemical attributes of Nigerian natural honey from honeybees. *Pakistan Journal of Biological Sciences*, 15(21), pp. 1027-1033.

Fletcher, J., 2020, *Why is ginger good for you?* [Online]. Available at: https://www.medicalnews today.com/articles/265990 [Accessed 21 January 2022]

Fredes, C. and Montenegro, G., 2006. Heavy metals and other trace element contents in Chilean honey. *Cienciae Investigación Agraria*, 33(1), pp. 50-58.

Ghasemzadeh, A., Jaafar, H. Z. and Rahmat, A., 2010. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Molecules*, 15(6), pp. 4324–4333.

Gheldof, N., Wang, X. H. and Engeseth, N. J., 2002. Identification and quantification of antioxidant components of honeys from various floral sources. *Journal of Agricultural and Food Chemistry*, 50(21), pp. 5870-5877.

Gleiter, R. A., Horn, H. and Isengard, H. D., 2006. Influence of type and state of crystallisation on the water activity of honey. *Food Chemistry*, 96(3), pp. 441–445.

Glevitzky, M., Dumitrel, G.A., Perju, D. and Popa, M. , 2009. Studies regarding the use of preservatives on soft drinks stability. *Chemical and Pharmaceutical Bulletin*, 54, pp. 31-36.

Graf, B. L., Raskin, .I, Cefalu, W.T. and Ribnicky, D.M., 2010. Plant derived therapeutics for the treatment of metabolic syndrome. *Current Opinion in Investigational Drugs*, 11, pp. 1107-1115.

Gonelimali, F. D. et al., 2018. Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Frontiers in Microbiology*, 9, pp. 39-49.

González, M. L. et al., 2005. Multivariate correlation between colour and mineral composition of honeys and by their botanical origin. *Journal of Agricultural and Food Chemistry*, 53(7), pp. 2574–2580.

Gupta, S.K. and Sharma, A., 2014. Medicinal properties of *Zingiber officinale* Roscoe - a review. *IOSR Journal of Pharmacy and Biological Sciences*, 9, pp. 124-129

Hazra, B., Biswas, S. and Mandal, N., 2008. Antioxidant and free radical scavenging activity of *Spondias pinnata*. *BMC Complementary and Alternative Medicine*, 8, pp. 63.

Hegazi, A. G. and Abd El-Hady, F. K., 2009. Influence of honey on the suppression of human low density lipoprotein (LDL) peroxidation (in vitro). *Evidence-based Complementary and Alternative Medicine*, 6(1), pp. 113–121.

Heredia, F.J., González-Miret, M.L., Meléndez-Martínez, A.J. and Vicario, I.M., 2013. Instrumental assessment of the sensory quality of juices. In: Kilcast, D. (eds.). *Instrumental Assessment of Food Sensory Quality, Sensory Quality*. Sawston: Woodhead Publishing, pp. 565-610e.

Imtara, H., Elamine, Y. and Lyoussi, B., 2018. Physicochemical characterization and antioxidant activity of Palestinian honey samples. *Food Science and Nutrition*, 6, pp. 2056- 2065.

Jasna, Č. et al., 2014. Evaluation of phenolic content, antioxidant activity and sensory characteristics of Serbian honey-based product. *Industrial Crops and Products*, 62, pp. 1-7.

Jimenez, M., Beristain, C.I., Azuara, E., Mendoza, M.R. and Pascual, L.A., 2016. Physicochemical and antioxidant properties of honey from *Scaptotrigona mexicana* bee. *Journal of Apicultural Research*, 55(2), pp. 151-160.

Kaakeh, W. and Gadelhak, G. G. (2005). Sensory evaluation and chemical analysis of *Apis mellifera* honey from the Arab Gulf region. *Journal of Food and Drug Analysis*, 13(4), pp. 331–337.

Kadri, S. M., Zaluski, R., Lima, G. P. P., Mazzafera, P. and Orsi, R. O., 2016. Characterization of *Coffea arabica* monofloral honey from Espírito Santo, Brazil. *Food Chemistry*, 203, pp. 252-257.

Kamal, M.A. and Klein, P., 2011. Determination of sugars in honey by liquid chromatography. *Saudi Journal of Biological Sciences*, 18(1), pp. 17-21.

Kasilingam T. et al., 2018. A review on in vitro regeneration of ginger: Tips and highlights. *European Journal of Medicinal Plants*, 23, pp. 1–8.

Kaskoniene, V., Venskutonis, P. and Ceksteryte, V., 2010. Carbohydrate composition and electrical conductivity of different origin honeys from Lithuania. *LWT - Food Science and Technology*, 43 (5), pp. 801-807.

Khalil, M. et al., 2012. Physicochemical and antioxidant properties of Algerian honey. *Molecules*, 17(9), pp. 11199-11215.

Koochak, H., Seyyednejad, S.M. and Motamedi, H., 2010. Preliminary study on the antibacterial activity of some medicinal plants of Khuzestan (Iran). Asian Pacific Journal of Tropical Medicine, 3(3), pp. 180-184.

Lee, H. et al., 2014. Physicochemical properties and antioxidant capacities of different parts of ginger (*Zingiber officinale* Roscoe). *Journal of the Korean Society of Food Science and Nutrition*, 43(9), pp. 1369-1379.

Lee, J., Koo, N. and Min, D. B., 2004. Reactive oxygen species, aging, and antioxidative nutraceuticals. *Comprehensive Reviews in Food Science and Food Safety*, 3 (1), pp. 21-33.

Life Science, 2013, *What Is Honey?* [Online]. Available at: https://www.livescience.com/37611-what-is-honey-honeybees.html [Accessed 22 January 2022].

Mallikarjuna, K., Sahitya, C. P., Sathyavelu, R. K. and Rajendra, W., 2008. Ethanol toxicity: Rehabilitation of hepatic antioxidant defense system with dietary ginger. *Fitoterapia*, 79, pp. 174–178.

Malu, S. P., Obochi, G. O., Tawo, E. N. and Nyong, B. E., 2009. Antibacterial activity and medicinal properties of ginger (*Zingiber officinale*). *Global Journal of Pure and Applied Sciences*, 15(3), pp. 365-368.

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.

Manikis, I. and Thrasivoulou, A., 2001. The relation of physicochemical characteristics of honey and the crystallization sensitive parameters. *Apiacta*, 36(3), pp. 106–112.

Martos, M.V., Navajas, Y.R., Fernandez-Lopez, J. and Perez-Alvarez, J.A., 2008. Functional properties of honey, propolis, and royal jelly. *Journal of Food Science*, 73 (9), pp. 117 – 124.

Mavric, E., Wittmann, S., Barth, G. and Henle, T., 2008. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium*) honeys from New Zealand. *Molecular Nutrition and Food Research*, 52(4), pp. 483-489.

Meo, S.A., Al-Asiri, S.A., Mahesar, A.L. and Ansari, M.J., 2017. Role of honey in modern medicine. *Saudi Journal of Biological Sciences*, 24(5), pp. 975-978.

Mishra, N. and Behal, K., 2010. Antimicrobial activity of some spices against selected microbes. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2 (3), pp. 187-196.

Mian, S.S., Upadhayay, S., Tajuddin and Naqvi, S.N., 2019. Physicochemical analysis of ginger (*Zingiber officinale* Rosc.) rhizome along with its TLC, *HPLC and HPTLC Profile*, 10(1), pp. 31-36.

Minden-Birkenmaier, B. and Bowlin, G., 2018. Honey-based templates in wound healing and tissue engineering. *Bioengineering*, 5(2), pp. 1-27.

Molan, P.C., 1992. The antibacterial activity of honey: 1. the nature of the antibacterial activity. *Bee World*, 73(1), pp. 5-28.

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

Moumeh, B. et al., 2020. Chemical analysis and sensory evaluation of honey produced by honeybee colonies fed with different sugar pastes. *Food Science and Nutrition*, 8, pp. 5823–5831.

Nafi, A., Ling, F. H., Bakar, J. and Ghazali, H. M., 2014. Partial characterization of an enzymatic extract from Bentong ginger (*Zingiber officinale* var. *Bentong*). *Molecules*, 19(8), pp. 12336–12348.

Nagai, L., Tanoue, Y., Kai, N. and Suzuki, N., 2012. Functional property of honey from *Echium vulgare*. *Food and Nutrition Sciences*, 3 (5), pp. 614-620.

National Chemical Emergency Centre, 2022, *Physico-chemical properties* [Online]. Available at: https://the-ncec.com/en/regulatory-compliance/environmental-chemistry-and toxicology/ expertise-in-environmental-chemistry-and-toxicology/physico-chemical-properties [Accessed 2 March 2022].

Nayaka, N. M. et al., 2020. Antioxidantand antibacterial activities of multiflora honey extracts from the Indonesian *Apis cerana* bee. *Journal of Taibah University Medical Sciences,* 15(3), pp. 211–217.

Nazeh, M.A. et al., 2015. Antioxidant, antibacterial activity, and phytochemical characterization of *Melaleuca cajuputi* extract. *BMC Complementary and Alternative Medicine,* 15(1), pp. 1-13.

Nebojša, N., Bursać, S. and Zlatković, B., 2020. Content of moisture, water activity, pH and electrical conductivity as indicator of honey quality from commercial stores and primary producers. *Proceedings of The International Symposium on Animal Science,* 4, pp. 235-240.

Ng, W.J. et al., 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 Medicines,* 11(2), pp. 315-318.

Nychas, G.J.E. and Skandamis, P.N., 2003. Antimicrobials from herbs and spices. In: Roller, S. (eds.). *Natural Antimicrobials for the Minimal Processing of Foods*. New York: CRC, pp. 95- 108.

Oddo L. P. and Piro, R., 2004. Main European unifloral honeys: descriptive sheets. *Apidologie,* 35 (1), pp. 38–81.

Okeola, F.O., Afolabi, B.T., Liadi, M.T. and Apo,G.S., 2015. Comparative study of physico-chemical properties of pure honey harvested from *Jatropha curcas*

plantation and honey fortified with moringa and ginger. *International Journal of Phytofuelsand Allied Sciences*, 4(1), pp. 81-92

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

Omoya, F. O. and Akharaiyi, F. C., 2012. Mixture of honey and ginger extract for antibacterial assessment on some clinical isolates. *International Research Journal of Pharmacy*, 2(5), pp. 127-132.

Park, M., Bae, J. and Lee, D. S., 2008. Antibacterial activity of [10]-gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria. *Phytotherapy Research*, 22(11), pp.1446-1449.

Pavithra, K. and Vadivukkarasi, S., 2015. Evaluation of free radical scavenging activity of various extracts of leaves from *Kedrostis foetidissima (Jacq.) Cogn. Food Science and Human Wellness*, 4(1), pp. 42-46.

Przygodzka, M. et al., 2014. Comparison of methods for evaluation of the antioxidant capacity and phenolic compounds in common spices. *LWT—Food Science and Technology*, 58(2), pp. 521-526.

Piana, M.L. et al., 2004. Sensory analysis applied to honey: State of the art. *Apidologie*, 35, pp. 26–S37.

Poolman, J. T. and Anderson, A. S., 2018. *Escherichia coli* and *Staphylococcus aureus*: leading bacterial pathogens of healthcare associated infections and bacteremia in older-age populations. *Expert Review of Vaccines*, 17 (7), pp. 607-618.

Purdie, J., 2019, *Is raw ginger acidic or alkaline?* [Online]. Available at: https://www.livestrong.com/article/528346-is-raw-ginger-acidic-or-alkaline/ [Accessed 21 March 2022].

Rahman, M.M., Allan, R. and Azirun, M.S., 2010. Antibacterial activity of propolis and honey against *Staphylococcus aureus* and *Escherichia Coli*. *African Journal of Microbiology Research*, 4, pp. 1872-1878.

Ramaa, C. S., Shirode, A. R., Mundada, A. S. and Kadam, V. J., 2006. Nutraceuticals an emerging era in the treatment and prevention of cardiovascular diseases. *Current Pharmaceutical Biotechnology*, 7, pp. 15–23.

Sahdeo, P. and Amit, K. T., 2015. Ginger and its constituents: Role in prevention and treatment of gastrointestinal cancer. *Gastroenterology Research and Practice*, 2015, pp. 1-11.

Sahney, M. and Kumar, A., 2017. Physiochemical and mineral analysis of honey samples from Varanasi District. *International Journal of Pharma and Bio Sciences*, 8, pp. 160-166.

Sajak, A. A. B., Azlan,A., Hamzah, H. and Abas, F., 2019. Phenolic content and α–glucosidase inhibitory activity of herbal mixture: Effect of processing technique and honey ratio. *Malaysian Journal of Medicine and Health Sciences*, 15 (2), pp. 58 -63.

Saludin, S.F. et al., 2019. Measuring consumers' preferences of stingless bee honey (meliponine honey) based on sensory characteristics. *International Food Research Journal*, 26(1), pp. 225235.

Sani, N. F. et al., 2014, Effect of the combination of gelam honey and ginger on oxidative stress and metabolic profile in streptozotocin-induced diabetic Sprague-Dawley rats. *BioMed research international*, 16, pp. 1-9.

Sharma, S. K. and Ajay, P. S., 2012. *In vitro* antioxidant and free radical scavenging activity of *Nardostachys jatamansi DC. Journal of Acupuncture and Meridian Studies*, 5(3), pp. 112-118.

Snowdon, J.A. and Cliver, D.O., 1996. Microorganisms in honey. *International Journal of Food Microbiology*, 31(1-3), pp. 1-26.

Sohaimy, S. A. E. Masry, S.H.D. and Shehata, M.G., 2015. Physicochemical characteristics of honey from different origins. *Annals of Agricultural Sciences,* 60 (2), pp. 279-287.

Straumite, E. et al., 2011. Physical and microbiological characteristics and antioxidant activity of honey bee pollen. *Applied. Science*. 12, pp. 3039- 3052.

Stoilova, I. et al., 2007. Antioxidant activity of a ginger extract (*Zingiber officinale*). *Food Chemistry,* 102 (3), pp. 764-770.

Suhaimi, M. Y., Mohamad, A. M. and Hani, M. F., 2014. Potential and viability analysis for ginger cultivation using fertigation technology in Malaysia. *International Journal of Innovation and Applied Studies*, 9 (1), pp. 421-427.

Syuhaidah, F., 2020. *Chemical variability in volatile oils of Bentong ginger: A chemometric study*. Master thesis, Universiti Malaya, Malaysia.

Tao, Z., Raffel, R.A., Souid, A.K. and Goodisman, J., 2009. Kinetic studies on enzyme-catalyzed reactions: oxidation of glucose, decomposition of hydrogen peroxide and their combination. *Biophysical Journal*, 96(7), pp. 2977-2988.

Technical University of Munich, 2018, *Pungent tasting substance in ginger reduces bad breath: How food ingredients affect our taste perception* [Online]. Available at: www.sciencedaily.com/releases/2018/07/180730120353.htm [Accessed 21 March 2022].

Terrab, A., Dıez, M.J. and Heredia, F.J., 2003. Palynological, physico-chemical and colour characterization of Moroccan honeys: I. River red gum (*Eucalyptus camaldulensis* Dehnh) honey. *International Journal of Food Science and Technology*, 38, pp. 379-386.

Thannickal, V. J. and Fanburg, B. L., 2000. Reactive oxygen species in cell signaling. *The American Journal of Physiology-Lung Cellular and Molecular Physiology,* 279(6), pp. 1005-1028.

Tohma, H. et al., 2017. Antioxidant activity and phenolic compounds of ginger (*Zingiber officinale* Rosc.) determined by HPLC-MS/MS. *Journal of Food Measurement and Characterization*, 11(2), pp.556–566.

Valgas C., De Souza, S.M. and Smânia, E.F.A., 2007. Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology*, 38, pp. 369–380.

Villazon, L., 2021, *How do bees make honey*? [Online]. Available at: https://www.sciencefocus.com/nature/how-do-bees-make-honey/ [Accessed 21 January 2022].

Wang, X.H., Gheldof, N. and Engeseth, N.J., 2004. Effect of processing and storage on antioxidant capacity of honey. *Journal of Food Science*, 69, pp. 96-101.
Weston, R.J. and Brocklebank, L.K., 1999. The oligosaccharide composition of some New Zealand honeys. *Food Chemistry*, 64(1), pp. 33-37.

Wilczyńska, A. and Ruszkowska, M., 2014. Water activity and colour parameters changes during storage of linden and buckwheat honeys. *Zeszyty Naukowe Akademii Morskiej w Gdyni* , 84, pp. 174—181.

Wu, J. et al., 2020. Quality comparison of multifloral honeys produced by *Apis cerana cerana, Apis dorsata* and *Lepidotrigona flavibasis*. *LWT*, 134, pp.220-225.

Yap, S. K., Chin, N. L., Yusof, Y. A. and Chong, K. Y., 2019. Quality characteristics of dehydrated raw Kelulut honey, *International Journal of Food Properties*, 22, pp. 556-571.

Yesiloglu, Y., Aydin, A. and Kilic, I., 2013. In vitro antioxidant activity of various extracts of ginger (*Zingiber officinale* L.) seed. *Asian Journal of Chemistry*, 25 (7), pp. 3573–3578.

Yusof, N. et al., 2013. The ferrous ion chelating assay of *Pandanus* extracts. *The Open Conference Proccedings Journal*, 4, pp. 155-156.

Yücel, Y. and Sultanog, P., 2013. Characterization of honeys from Hatay Region by their physicochemical properties combined with chemometrics. *Food Bioscience*, 1, pp. 16–25.

Zainol, M.I., Yusoff, K.M. and Yusof, M.Y.M., 2013. Antibacterial activity of selected Malaysian honey. *BMC Complementary and Alternative Medicine*, 13(1), pp. 1-10.

Zamora, M. C., Chirife, J., and Roldán, D., 2006. On the nature of the relationship between water activity and % moisture in honey. *Food Control*, 17(8), pp. 642–647.

Zhang, Y. Z et al., 2019. Authentication of *Apis cerana* honey and *Apis mellifera* honey based on major royal jelly protein 2 gene. *Molecules*, 24(2), p. 289.

Zhou, A. et al., 2009. Distinctive oxidative stress responses to hydrogen peroxide in sulfate reducing bacteria *Desulfovibrio vulgaris Hildenborough. TBD*, pp. 1 – 47.

Zhou, J. et al., 2012. Protective effects of buckwheat honey on DNA damage induced by hydroxyl radicals. *Food and Chemical Toxicology*, 50 (8), pp. 2766-2773.

APPENDICES

APPENDIX A

Figure A: Rainforest wild raw honey from Malaysia.

Figure B: Ginger Honey Bentong, the left one is added with 10% ginger nd the right one is added with 18% ginger.

APPENDIX B

Table A: List of chemicals and media with their respective manufacturers.

Chemicals/ Media	**Manufacturers**
β- Nicotinamide Adenine Dinucleotide Disodium Salt (Reduced form)	Nacalai Tesque, Japan
2-Deoxy-D-ribose	ACROS Organics, China
2-Thiobarbituric acid	Sigma-Aldrich, USA
3, 5-Dinitrosalicyclic acid	ACROS Organics, UK
Ampicillin	Bio Basic Canada Inc, Canada
D-glucose	SYSTERM, Malaysia
EDTA-2Na	DUKSAN Reagents, Korea
Ethyl Alcohol (95 %)	Chem Soln, India
FerroZine iron reagent hydrate	ACROS Organics, Austria
Folin and Ciocalteu's phenol reagent	Chem Soln, India
Gallic acid	Bio Basic Canada Inc, Canada
Hydrogen peroxide, 30 - 32 %	QRec, Singapore
Iron (III) Chloride Hexahydrate	Nacalai Tesque, Japan
Iron (II) sulfate -7- hydrate	Bendosen Laboratory Chemicals, Malaysia

Table A (continue): List of chemicals and media with their respective manufacturers.

Chemicals/ Media	Manufacturers
L-ascorbic acid	GENE Chem, France
MacConkey agar	Chem Soln, India
Mueller Hinton agar	HiMedia Laboratories, India
Nitro Blue Tetrazolium Tablet	Sigma-Aldrich, USA
Nutrient agar	Merck KGaA, Germany
Phenazine methosulfate	Chem Soln, India
Potassium phosphate (Dibasic)	Bio Basic Canada Inc, Canada
Potassium phosphate (Monobasic)	Bio Basic Canada Inc, Canada
Potassium sodium tartrate tetrahydrate	R&M Chemicals, UK
Sodium carbonate	R&M Chemicals, UK
Sodium chloride	Chem Soln, India
Sodium hydroxide	R&M Chemicals, UK
Sodium sulfite anhydrous	Bendosen Laboratory Chemicals, Malaysia
Trichloroacetic acid	Fisher Scientific, USA
Tris base	Fisher Scientific, USA

Table B: Equipment and labware used with their respective manufacturers.

Equipment/Labware	Manufacturers
Aluminium foil	Diamond, China
Analytical balance	Kern & Sohn, Germany
Beaker (50 mL, 100 mL, 250 mL, 500 mL and 1 L)	GQ, Malaysia
Bunsen Burner	HmbG Eco, Malaysia
CM-600d Spectrophotometer	Konica Minolta Sensing Americas, USA
Cotton swab	Biomedia, Singapore
Falcon tube (15 mL and 50 mL)	NEST Biotechnology Co. Ltd., USA
Flat-bottomed 96 well microplate	NEST Biotechnology Co. Ltd., USA
FLUOstar Omega microplate reader	Labtech, Germany
Hot plate & MagneticStirrer	LMS, Japan
Incubator	Memmert GmbH + Co.KG, Germany
Laminar flow cabinet	Streamline Laboratory Products, Singapore
Measuring cylinder (5 mL, 50 mL, 100 mL, 250 mL, 500 mL)	Bomex, China
Micropipette set	Hercuvan Lab Systems Inc., USA
Micropipette tips	NEST Biotechnology Co. Ltd., USA
Multi parameter PCSTestrTM 35	Oakton, USA
Paper cups	Shamoji, Japan
Parafilm	Bemis, USA

Table B (continue): Equipment and labware used with their respective manufacturers.

Equipment/Labware	Manufacturers
Petri dish	NEST Biotechnology Co. Ltd., USA
pH meter	Sartorius, Germany
Refractometer	ATAGO, USA
Schott bottle	Duran, Germany
SP-V1000 Spectrophotometer	DLAB Scientific, China
Stopwatch	Canon, Japan
Syringe filter (0.45 um)	Membrane Solutions, China
Vortex mixture	Gemmy Industrial Corp., Taiwan
Water activity meter	Novasina, India
Water bath	Memmert GmbH + Co.KG, Germany
Weighing machine	Copens Scientific (M) Sdn. Bhd., Malaysia
WPA Lightwave II UV Spectrophotometer	Biochrom, U

APPENDIX C

Reagent and media preparations

Ampicillin solution (10 µg/mL)

Ampicillin solution was prepared by adding approximately 0.001 g of ampicillin powder into 100 mL of autoclaved distilled water.

Ascorbic acid solution (1.8mM)

Ascorbic acid solution was prepared by adding approximately 0.03566 g of ascorbic acid powder into 100 mL of distilled water.

Deoxyribose solution (50mM)

Deoxyribose solution was prepared by adding approximately 0.6707 g of deoxyribose powder into distilled water and then topped up the mixture to a final volume of 100 mL.

Ferric chloride solution (3.2mM)

Ferric chloride solution was prepared by adding approximately 0.0519 g of ferric chloride powder into 100 mL of distilled water.

Ferrous sulphate solution (1mM)

Ferrous sulphate solution was prepared by adding approximately 0.0278 g of ferrous sulphate heptahydrate powder into 100 mL of distilled water.

Ferrozine solution (0.25mM)

Ferrozine solution was prepared by adding approximately 0.13 g of ferrozine powder into 1 L of distilled water.

Gallic acid standard solution (100 µg/mL)

Gallic acid standard solution was prepared by adding approximately 0.01 g of gallic acid into 100 mL of distilled water.

Glucose standard solution (1000 µg/mL)

Glucose standard solution was prepared by adding approximately 0.05 g of glucose into 50 mL of distilled water.

Hydrogen peroxide (H_2O_2) solution (50mM)

H_2O_2 solution was prepared by adding approximately 0.153 mL of 30% H_2O_2 into 100 mL of distilled water.

Dipotassium phosphate (K_2HPO_4)(1M)

K_2HPO_4 solution was prepared by adding approximately 17.418 g of K_2HPO_4 powder into 100 mL of distilled water.

Potassium dihydrogen phosphate (KH_2PO_4)(1M)

KH_2PO_4 solution was prepared by adding approximately 13.609 g of KH_2PO_4 powder into 100 mL of distilled water.

NADH (2.34mM)

NADH solution was prepared by adding approximately 0.0166 g of NADH powder into the 10 mL of Tris buffer.

Na_2EDTA solution (1mM)

Na_2EDTA solution was prepared by dissolving adding approximately 0.0372 g of Na_2EDTA powder into 100 mL of distilled water.

Nitroblue tetrazolium (NBT) stock solution

NBT solution was prepared by adding 10 mg of NBT tablet into 1 mL of distilled water.

Nitroblue tetrazolium (NBT) (0.78mM)

NBT solution was prepared by adding approximately 64 µL of NBT stock solution into 936 µL of distilled water and then topped up to 1000 µL.

Phenazine methosulfate (0.06mM)

Phenazine methosulfate solution was prepared by adding approximately 0.0184 g of PMS powder into 1 L of distilled water.

Phosphate buffer (100mM, pH 7.4)

Phosphate buffer was prepared by adding approximately 40.1 mL of K_2HPO_4 into 9.9 mL of KH_2PO_4 and 450 mL of distilled water.

Rochelle salt [40% (w/v)]

Rochelle salt was prepared by adding approximately 2 g of potassium sodium tartrate tetrahydrate powder into 5 mL of distilled water.

Sodium carbonate solution [10% (w/v)]

Sodium carbonate solution was prepared by adding approximately 5 g of sodium carbonate into 50 mL of distilled water.

Sodium hydroxide (NaOH) solution (1.25%)

NaOH solution was prepared by adding 1.316 g of NaOH pellet in 100 mL of distilled water.

Sugar solution (43% fructose, 28% glucose, 2% sucrose)

Sugar solution was prepared by adding approximately 43 g of fructose, 28 g of glucose and 2 g of sucrose into 100 mL of warm distilled water.

Thiobarbituric acid (TBA) solution (5% w/v)

TBA solution was prepared by adding approximately 0.5 g of TBA powder into 1.25% NaOH with volume of 10 mL.

Trichloroacetic acid (TCA) solution (10% w/w)

TCA solution was prepared by adding approximately 1 g of TCA powder into 10 mL of distilled water.

Tris buffer (0.1 M, pH 8)

Tris buffer was prepared by adding approximately 0.605 g of Tris base into distilled water with a final volume of 50 mL.

APPENDIX D

1. Be in correct physical and mental condition
2. Know the score card
3. Know the defects and the range of probable intensities
4. For some foods and beverages, it is useful to observe aroma immediately after opening the sample container
5. Taste a sufficient volume (Be professional—not timid!)
6. Pay attention to the sequence of flavors
7. Rinse, occasionally, as the situation and product type warrant
8. Concentrate. Think about your sensations and block out all other distractions
9. Do not be too critical. Also, do not gravitate to the middle of the scale
10. Do not change your mind. Often the first impression is valuable, especially for aromas
11. Check your scoring after the evaluation. Get feedback on how you are doing
12. Be honest with yourself. In the face of other opinions, "stick to your guns"
13. Practice. Experience and expertise come slowly. Be patient
14. Be professional. Avoid informal lab banter and ego trips Insist on proper experimental controls—watch out for benchtop "experiments"
15. Do not smoke, drink, or eat for at least 30 min before participation
16. Do not wear perfume, cologne, aftershave, etc. Avoid fragranced soaps and hand lotions

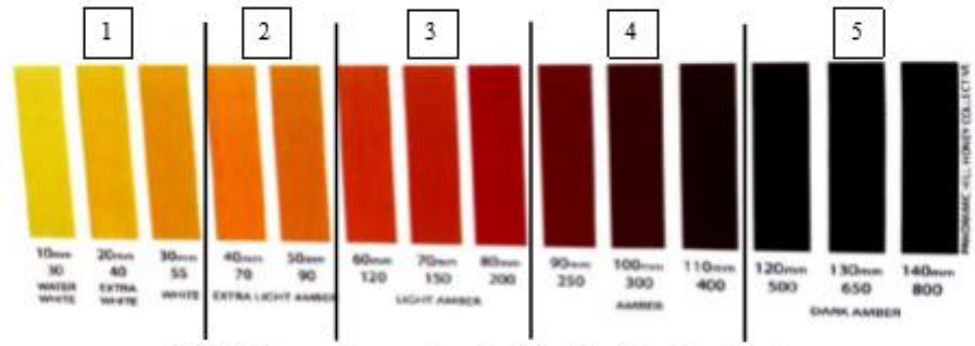
Modified from Nelson and Trout (1964)

Figure A: Guidelines for panelist.

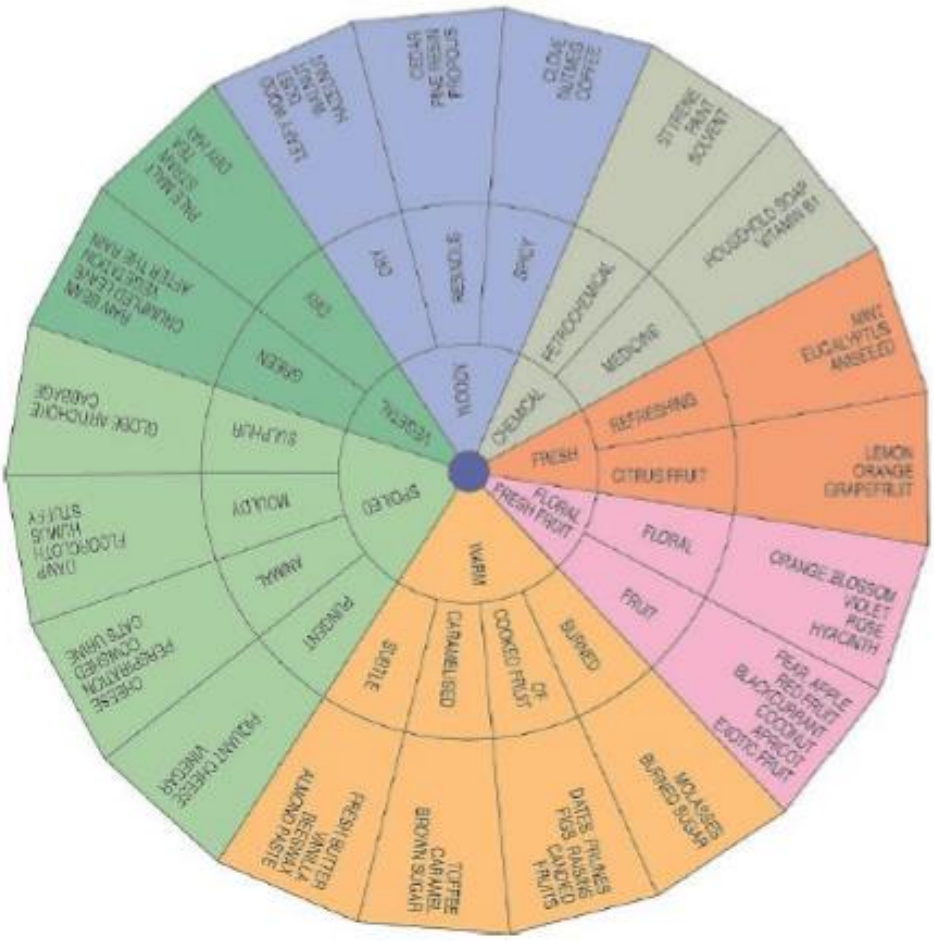
Attribute	Description	Type of rating	Presentation and evaluation
Visual	Colour intensity Viscosity	*5-point scaling	-samples will be presented in a transparent glass vial -colour intensity reference will be provided -viscosity can be evaluated by rotating the glass vial
Olfactory	Woody Chemical Fresh Floral fresh fruit Warm Spoiled Vegetal	Yes/No	-coffee bean will be provided to relax smell Evaluation steps: - the vial will be uncapped for evaluation -evaluate odour immediately after uncapping (1st sniff) and after 10s /20s (2nd sniff)
Taste	Woody Chemical Fresh Floral fresh fruit Warm Spoiled Vegetal Astringency Refreshing sensation	Yes/No	-disposable plastic spoon will be given -water will be provided to clean palate between samples Evaluation steps: -deep inhale, hold the breath, use the disposable spoon to place honey on tongue, allow to dissolve before swallow, keep mouth closed, exhale through nose -wait at least 1 or 2 min lapse before 2nd tasting -rate the 'Aftertaste' intensities 1 min after the product is swallowed
	Sweetness Sourness Saltiness Bitterness Persistence After taste	*5-point scaling	
Texture	Adhesiveness	*5-point scaling	
Acceptability	How much you like or dislike	Hedonic 5-point scaling (1 = dislike very much, 2 = dislike slightly, 3 = neither like nor dislike, 4 = like slightly, 5 = like very much)	

*5-point scaling
1 = Very weak
2 = Weak
3 = Moderate
4 = Strong
5 = Very strong

Figure B: The flow of sensory evaluation.



USDA Honey colour grading chart for colour intensity attribute
(For colour intensity rating: 1-white; 2-extra light amber; 3-light amber; 4-amber; 5-dark amber)



Odour and aroma wheel for olfactory and taste attributes

Figure C: Guidelines for colour intensity rating and listing of the attributes.

Visual attributes	
Colour intensity	Degree of amber colour (varying from white to dark amber)
Viscosity	Force required to move the honey by rotation
Aroma attributes	
Spoiled	Associated with pungent, animals or degradation (mold, urine).
Floral, fruity	Associated with different flowers and fruits
Fresh	Associated with citrus food or mint
Vegetal	Associated with gardens, green notes, dry leaves, and wood
Warm	Associated with foods characterized by their sweet smell and taste.
Chemical	Not associated with food, it is characterized by its aggressiveness (smoked, phenolic, sulfuric).
Woody	Aromatics associated with dry fresh-cut wood: bark, cinnamon, dust.
Taste	
Sweetness	Sensation produced by products that contain sugars such as sucrose and fructose.
Sourness	Sensation produced by products that contain acids, such as citrus.
Saltiness	Sensation produced by products that contain salts, such as sodium chloride
Bitterness	Sensations produced by products such as caffeine.
Persistence	Feeling similar to what is perceived while the product was in the mouth and while continuing over a period of time measurable.
Astringency	Organoleptic property of pure substances or mixture which produce an astringent sensation.
Refreshing	Sensation of freshness and cooling in the oral cavity (similar to that produced by mint)
Texture attributes	
Adhesiveness	Ability of honey to stick to the teeth and oral cavity.

Figure D: Description for attributes.

Questionnaire for Flavour Profile Test

Panel Number: _____ Date: _____

Instruction:

Taste these samples one at a time and indicate the intensity of all the attributes (character notes) in the space provided, using the following scale:

1 = Very weak 2 = Weak 3 = Moderate 4 = Strong 5 = Very strong

Or **circle** the answer yes/no.

Attribute	Sample			
Visual				
Color intensity				
Viscosity				
Olfactory				
Woody	yes / no	yes / no	yes / no	yes / no
Chemical	yes / no	yes / no	yes / no	yes / no
Fresh	yes / no	yes / no	yes / no	yes / no
Floral fresh fruit	yes / no	yes / no	yes / no	yes / no
Warm	yes / no	yes / no	yes / no	yes / no
Spoiled	yes / no	yes / no	yes / no	yes / no
Vegetal	yes / no	yes / no	yes / no	yes / no
Taste				
Woody	yes / no	yes / no	yes / no	yes / no
Chemical	yes / no	yes / no	yes / no	yes / no
Fresh	yes / no	yes / no	yes / no	yes / no
Floral fresh fruit	yes / no	yes / no	yes / no	yes / no
Warm	yes / no	yes / no	yes / no	yes / no
Spoiled	yes / no	yes / no	yes / no	yes / no
Vegetal	yes / no	yes / no	yes / no	yes / no
Astringency	yes / no	yes / no	yes / no	yes / no
Refreshing	yes / no	yes / no	yes / no	yes / no
Sweetness				
Sourness				
Saltiness				
Bitterness				
Persistence				
After taste				
Texture				
Adhesiveness				

1 = dislike very much 2 = dislike slightly 3 = neither like nor dislike
4 = like slightly 5 = like very much

Acceptability				

Figure E: Score sheet.

APPENDIX E

Table A: Gallic acid standard solution preparation

	Concentration of gallic acid standard solution (µg/mL)				
	20	40	60	80	100
100 µg/mL of gallic acid solution (mL)	2	4	6	8	10
Distilled water (mL)	8	6	4	2	0
Final volume (mL)	10	10	10	10	10

Table B: Glucose standard solution preparation

	Concentration of glucose acid standard solution (µg/mL)				
	20	40	60	80	100
100 µg/mL of glucose standard solution (mL)	2	4	6	8	10
Distilled water (mL)	8	6	4	2	0
Final volume (mL)	10	10	10	10	10

APPENDIX F

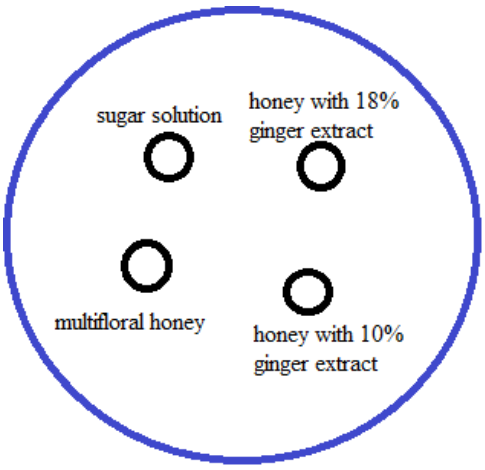


Figure A: Label of wells for each honey samples against *Escherichia coli* ATCC 25922.

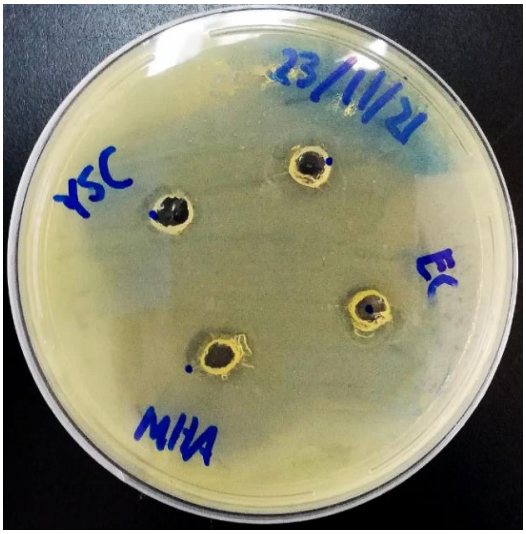


Figure B: Example of agar well diffusion assay of ORI, H10, H18 and sugar solution against *Escherichia coli* ATCC 25922. The assay was performed in triplicates.

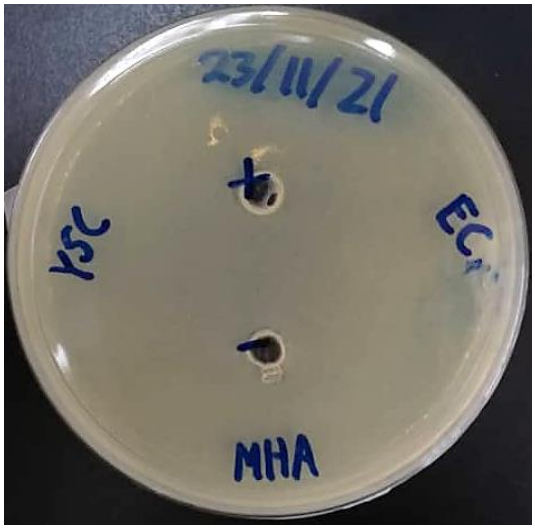


Figure C: Example of agar well diffusion assay of positive and negative control against *Escherichia coli* ATCC 25922. +: Positive control (10 µg/mL ampicillin solution); -: Negative control (distilled water).

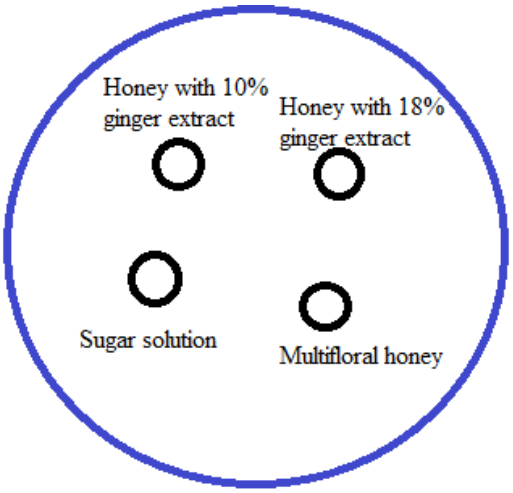


Figure D: Label of wells for each honey samples against *Staphylococcus aureus* ATCC 25923.

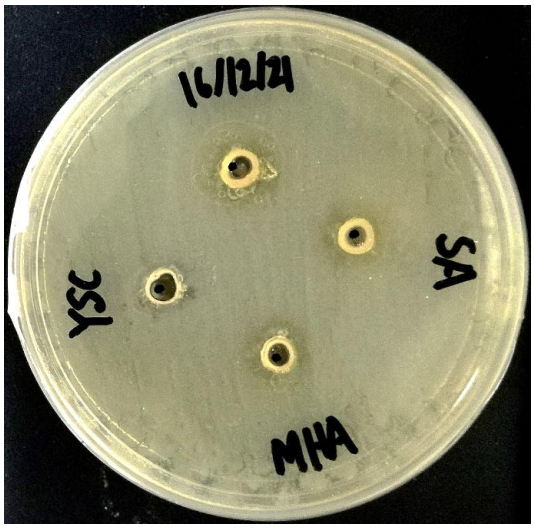


Figure E: Example of agar well diffusion assay of ORI, H10, H18 and sugar solution against *Staphylococcus aureus* ATCC 25923. The assay was performed in triplicates.

Figure F: Example of agar well diffusion assay of positive and negative control against *Staphylococcus aureus* ATCC 25923. +: Positive control (10 µg/mL ampicillin solution); -: Negative control (distilled water).

APPENDIX G

ANOVA test for each assay

The comparison of results between base honey samples and ginger honey samples.

Table A: Statistical analysis for colour intensity of honey samples.

Sample (I)	Sample (J)	Mean Difference (I-J)	Significant
ORI	H10	-0.09*	0.000
	H18	-0.06*	0.000
H10	ORI	0.90*	0.000
	H18	0.03*	0.009
H18	ORI	0.06*	0.000
	H10	-0.03*	0.009

* The mean difference is significant at the 0.05 level.

Table B: Statistical analysis for pH of honey samples.

Sample (I)	Sample (J)	Mean Difference (I-J)	Significant
ORI	H10	-0.31*	-6.000
	H18	-0.35*	-6.000
H10	ORI	0.31*	-6.000
	H18	-0.04*	0.004
H18	ORI	0.35*	-6.000
	H10	0.04*	0.004

* The mean difference is significant at the 0.05 level.

Table C: Statistical analysis for water activity of honey samples.

Sample (I)	Sample (J)	Mean Difference (I-J)	Significant
ORI	H10	0.00	0.349
	H18	-0.01*	0.002
H10	ORI	0.00	0.349
	H18	-0.01*	0.003
H18	ORI	0.01*	0.002
	H10	0.01*	0.003

* The mean difference is significant at the 0.05 level.

Table D: Statistical analysis for electrical conductivity of honey samples.

Sample (I)	Sample (J)	Mean Difference (I-J)	Significant
ORI	H10	-154.70*	-7.000
	H18	-242.30*	-7.000
H10	ORI	154.70*	-7.000
	H18	-87.60*	-6.000
H18	ORI	242.30*	-7.000
	H10	87.60*	-6.000

* The mean difference is significant at the 0.05 level.

Table E: Statistical analysis for total sugar content of honey samples.

Sample (I)	Sample (J)	Mean Difference (I-J)	Significant
ORI	H10	-1.30	-
	H18	-1.40	-
H10	ORI	1.30	-
	H18	-2.70	-
H18	ORI	1.40	-
	H10	-2.70	-

* The mean difference is significant at the 0.05 level.

Table F: Statistical analysis for reducing sugar content of honey samples.

Sample (I)	Sample (J)	Mean Difference (I-J)	Significant
ORI	H10	-2.52*	0.010
	H18	-4.80*	0.001
H10	ORI	-2.52*	0.010
	H18	-2.28*	0.015
H18	ORI	4.80*	0.001
	H10	2.28*	0.015

* The mean difference is significant at the 0.05 level.

Table G: Statistical analysis for moisture content of honey samples.

Sample (I)	Sample (J)	Mean Difference (I-J)	Significant
ORI	H10	1.30	-
	H18	-1.40	-
H10	ORI	-1.30	-
	H18	-2.70	-
H18	ORI	1.40	-
	H10	2.70	-

Table H: Statistical analysis for zone of inhibition diameter of honey samples for *Escherichia coli*.

Sample (I)	Sample (J)	Mean Difference (I-J)	Significant
ORI	H10	-0.08	0.238
	H18	-0.15	0.081
H10	ORI	0.08	0.238
	H18	-0.07	0.116
H18	ORI	0.15	0.081
	H10	0.07	0.116

Table I: Statistical analysis for zone of inhibition diameter of honey samples for *Staphylococcus aureus*.

Sample (I)	Sample (J)	Mean Difference (I-J)	Significant
ORI	H10	-0.05	0.548
	H18	-0.13	0.091
H10	ORI	0.05	0.548
	H18	-0.08	0.279
H18	ORI	0.13	0.091
	H10	0.08	0.279

* The mean difference is significant at the 0.05 level.

Table J: Statistical analysis for total phenolic content of honey samples.

Sample (I)	Sample (J)	Mean Difference (I-J)	Significant
ORI	H10	-16.89*	-5.000
	H18	-25.76*	-6.000
H10	ORI	16.89*	-5.000
	H18	-8.87*	-6.000
H18	ORI	25.76*	-6.000
	H10	8.87*	-6.000

* The mean difference is significant at the 0.05 level.

Table K: Statistical analysis for hydroxyl radical scavenging activity of honey samples.

Sample (I)	Sample (J)	Mean Difference (I-J)	Significant
ORI	H10	-1.43	0.054
	H18	-2.17*	0.006
H10	ORI	1.43	0.054
	H18	-0.74	0.247
H18	H10	0.74	0.247
	ORI	2.17*	0.006

* The mean difference is significant at the 0.05 level.

Table L: Statistical analysis for superoxide anion scavenging activity of honey samples.

Sample (I)	Sample (J)	Mean Difference (I-J)	Significant
ORI	H10	-24.47*	0.000
	H18	-29.28*	-7.000
H10	ORI	24.47*	0.000
	H18	-4.81*	0.044
H18	ORI	29.28*	-7.000
	H10	4.81*	0.044

* The mean difference is significant at the 0.05 level.

Table M: Statistical analysis for iron chelating activity of honey samples.

Sample (I)	Sample (J)	Mean Difference (I-J)	Significant
ORI	H10	-11.68*	0.017
	H18	-19.64*	-5.000
H10	ORI	11.68*	0.017
	H18	-7.96	0.065
H18	ORI	19.64*	-5.000
	H10	7.96	0.065

* The mean difference is significant at the 0.05 level.

Turnitin Originality Report

Processed on: 20-Apr-2022 13:41 +08
ID: 1815217227
Word Count: 15173
Submitted: 1

FYP Thesis First Submission By See Cheng YIP

Ng Wen Jie

EKY
Ee Kah Yaw
22 April 2022

Similarity Index	Similarity by Source
13%	Internet Sources: 9% Publications: 9% Student Papers: 3%

1% match (Internet from 01-May-2021) http://eprints.utar.edu.my/2940/1/BM%2D2017%2DNCW%2D1305016.pdf
1% match (publications) Mohammed Moniruzzaman, Md Ibrahim Khalil, Siti Amrah Sulaiman, Siew Hua Gan. "Physicochemical and antioxidant properties of Malaysian honeys produced by Apis cerana, Apis dorsata and Apis mellifera", BMC Complementary and Alternative Medicine, 2013
1% match (Internet from 09-Dec-2014) http://www.science.gov/topicpages/s/strong+antioxidant+activities.html
1% match () Wen-Jie Ng, Nam-Weng Sit, Peter Aun-Chuan Ooi, Kah-Yaw Ee, Tuck-Meng Lim. "The Antibacterial Potential of Honeydew Honey Produced by Stingless Bee (Lagainst Antibiotic Resistant Bacteria", Antibiotics
< 1% match (Internet from 24-Oct-2017) http://eprints.utar.edu.my/2297/1/BM-2016-1202692-1.pdf
< 1% match (Internet from 27-Jul-2021) http://eprints.utar.edu.my/2274/1/BM%2D2016%2D1204337%2D1.pdf
< 1% match (Internet from 21-Jul-2020) http://eprints.utar.edu.my/2296/1/BM-2016-1300054-1.pdf
< 1% match (Internet from 22-Jan-2022) https://cyberleninka.org/article/n/572067
< 1% match (Internet from 13-Feb-2022) https://cyberleninka.org/article/n/1211963
< 1% match (Internet from 19-Mar-2019) https://link.springer.com/article/10.1007%2Fs12010-015-1946-x
< 1% match (Internet from 15-Jul-2020) https://link.springer.com/article/10.1007/s13197-019-03728-3?code=f9ee74bf-5719-4a05-ac68-b8bbf97801f3&error=cookies_not_supported
< 1% match (Internet from 13-Nov-2021) https://link.springer.com/article/10.1208/s12249-015-0386-x?code=1e4ec9bb-55dd-4c3f-9afe-7885efe8b6b9&error=cookies_not_supported
< 1% match (Internet from 16-Jul-2020) https://link.springer.com/chapter/10.1007%2F978-1-4614-4310-0_4
< 1% match (Internet from 14-Feb-2022) https://link.springer.com/article/10.1007/s11694-021-00988-6?code=425dae0a-8d42-4f64-be26-c9dec3fa34db&error=cookies_not_supported
< 1% match (Internet from 30-Dec-2019) https://link.springer.com/article/10.1007%2Fs11694-019-00167-8
< 1% match (publications) Dan Dan Qian, Yun Tao Li, Yang Shu, Yao Dong Gu, Yan Zhang, Jan Awrejcewicz, Xue Wen Jia. "Dynamic Balance Analysis under Different Lower Extremities Tortuosity", Journal of Biomimetics, Biomaterials and Biomedical Engineering, 2017
< 1% match (publications) Maria Lucia Piana. "Sensory analysis applied to honey: state of the art", Apidologie, 2004
< 1% match (Internet from 23-Dec-2020) https://www.mdpi.com/2079-6382/9/12/871/htm
< 1% match (Internet from 20-Feb-2021) https://www.tmbfs.org/authors/
< 1% match (Internet from 22-Aug-2021) https://euacademic.org/UploadArticle/4173.pdf
< 1% match (publications) Ntsoaole, Kebede, P.A. Subramanian, and Gebrekidan Mebrahtu. "Physicochemical analysis of Tigray honey: An attempt to determine major quality markers of honey", Bulletin of the Chemical Society of Ethiopia, 2012.

Universiti Tunku Abdul Rahman			
Form Title : Supervisor's Comments on Originality Report Generated by Turnitin for Submission of Final Year Project Report (for Undergraduate Programmes)			
Form Number: FM-IAD-005	Rev No.: 1	Effective Date: 3/10/2019	Page No.: 1of 1

FACULTY OF SCIENCE

Full Name(s) of Candidate(s)	YIP SEE CHENG
ID Number(s)	18ADB03172
Programme / Course	BACHELOR OF SCIENCE (HONS) BIOMEDICAL SCIENCE
Title of Final Year Project	INFLUENCE OF ADDITION OF GINGER ON SENSORY QUALITY, PHYSICOCHEMICAL PROPERTIES AND BIOACTIVITIES OF MALAYSIAN MULTIFLORAL HONEY

Similarity	**Supervisor's Comments (Compulsory if parameters of originality exceeds the limits approved by UTAR)**
Overall similarity index: 13 % **Similarity by source** Internet Sources: 9 % Publications: 9 % Student Papers: 3 %	
Number of individual sources listed of more than 3% similarity: 0	

Parameters of originality required and limits approved by UTAR are as follows:

(i) Overall similarity index is 20% and below, and

(ii) Matching of individual sources listed must be less than 3% each, and

(iii) Matching texts in continuous block must not exceed 8 words

Note: Parameters (i) – (ii) shall exclude quotes, bibliography and text matches which are less than 8 words.

Note Supervisor/Candidate(s) is/are required to provide softcopy of full set of the originality report to Faculty/Institute

Based on the above results, I hereby declare that I am satisfied with the originality of the Final Year Project Report submitted by my student(s) as named above.

Signature of Supervisor
Name: NG WEN JIE

Date: 20/4/2022

Signature of Co-Supervisor
Name: EE KAH YAW

Date: 20/4/2022