SENSORY EVALUATION, PHYSICOCHEMICAL PROPERTIES AND BIOACTIVITIES OF *Apis cerana* HONEY WITH GINGER

By

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ABSTRACT

SENSORY EVALUATION, PHYSICOCHEMICAL PROPERTIES AND BIOACTIVITIES OF *Apis cerana* HONEY WITH GINGER

Chin Hui Yee

There is a rise of consumer demand for a natural, healthy and saporous product, and this study presents the investigation on an innovative honey-based product which is ginger honey. The influence of ginger on the sensory quality of ginger honey in terms of visual, olfactory, taste, texture and acceptability were assessed. The physicochemical properties analyzed in this study were colour intensity, water activity, electrical conductivity, sugar content, moisture content, pH and reducing sugar content. The antioxidant capacities of the ginger honey were assessed using total phenolic content, iron chelating activity, superoxide anion radical scavenging activity and hydroxyl radical scavenging activity. Agar well diffusion assay was performed to evaluate the antibacterial activity of ginger honey. Generally, the addition of ginger increased woody, fresh, astringency and bitterness attributes but decreased sweetness, floral fresh fruit, warm, persistence and aftertaste attributes of the honey, however, the acceptability of ginger honey was reduced. Furthermore, the addition of ginger increased the colour intensity, moisture content, water activity, electrical conductivity, pH and reducing sugar content but decreased the total sugar content. Moreover, the addition of ginger had a significant contribution on the increment of total phenolic content, iron

chelating activity, superoxide anion radical scavenging activity and hydroxyl radical scavenging activity of honey. Lastly, the ginger honey had a significant increment of antibacterial action on *Enterococcus faecalis* compared to the original honey. Contrarily, the ginger honeys did not cause significant increment of antibacterial effect against *Pseudomonas aeruginosa*. Overall, the addition of ginger had a negative impact on the overall acceptability due to altered aroma and taste attributes, caused changes on the physicochemical properties and enhanced the antioxidant capacities and antibacterial activities of honey.

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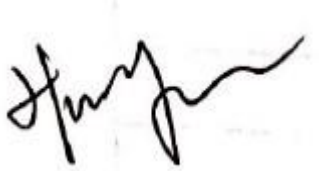
DECLARATION

I hereby declare that this final year 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.



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APPROVAL SHEET

This final year project report entitled "SENSORY EVALUATION, PHYSICOCHEMICAL PROPERTIES AND BIOACTIVITIES OF *Apis cerana* HONEY WITH GINGER" was prepared by CHIN HUI YEE and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Biomedical Science at Universiti Tunku Abdul Rahman.

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PERMISSION SHEET

It is hereby certified that **CHIN HUI YEE (ID No: 17ADB06004)** has completed this final year project report / thesis entitled **"SENSORY EVALUATION, PHYSICOCHEMICAL PROPERTIES AND BIOACTIVITIES OF *Apis cerana* HONEY WITH GINGER"** under the supervision of Mr. Ng Wen Jie (Supervisor) from the Department of Allied Health Science, Faculty of Science, and Dr. Ee Kah Yaw (Co-supervisor) from the Department of Agriculture and Food Science, Faculty of Science.

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

Yours truly,

(CHIN HUI YEE)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AD	Anno Domini
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
Aw	Water activity
CFU	Colony-forming unit
°C	Degree celcius
DNSA	3,5-dinitrosalicylic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
DW	Dry weight
et al.	*et alia*
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalence
g/mL	Gram per milliliter
HMF	Hydroxymethylfurfural
µg	Microgram
µg/mL	Microgram per milliliter
µL	Microliter
µM	Micromolar
µS/cm	MicroSiemens per centimeter
mAU	Milli difference
MDA	Malondialdehyde
mg/kg	Milligram per kilogram
mg/mL	Milligram per milliliter
MH	Mueller-Hinton
MIC	Minimal inhibitory concentration
mM	Millimolar

NBT	Nitro blue tetrazolium
PMS/NADH	Phenazine methosulfate-nicotinamide adenine dinucleotide
ppm	Parts per million
%	Percentage
% RSA	Percentage radical scavenging activity
PBS	Phosphate-buffered saline
pH	Potential of hydrogen
RE	Rutin equivalent
rpm	Revolutions per minute
ROS	Reactive oxygen species
spp.	Species
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TE	Trolox equivalents
TPC	Total phenolic content
v/v	Volume per volume
w/v	Weight per volume

CHAPTER 1

INTRODUCTION

Honey is a sweet and viscous substance that is produced by honey bees and can be used or stored exactly as produced in nature (White, 1978). Due to its sensory properties (taste, smell, texture), nutritional values and medicinal qualities, it has been well accepted by consumers all around the world. The presence of active constituents in honey contributes to the antioxidant and antibacterial properties which results in health promoting properties such as to treat cough, improve digestion and boost immunity (Wilczyńska et al., 2017; Dżugan et al., 2018). Honey exhibits antioxidant characteristics due to the presence of antioxidant compounds such as phenolics acid, flavonoids, vitamins, enzymes and trace elements (Gheldof et al., 2002). In addition, low water activity, high sugar content, acidity and polyphenol content of honey are responsible for its antibacterial activity against some of the human pathogens such as *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* (Olaitan et al., 2007; Kwakman and Zaat, 2012; Aggad and Guemou, 2014).

Since ancient times, ginger has been recognised as a popular spice in food products with intense aroma and taste. The health benefits of ginger in managing conditions such as colds, nausea, cardiovascular disease and obesity are attributed to its phenolics compounds (Mao et al., 2019). Recently, ginger has been reported as a potential agent for anti-inflammatory, anticancer and antioxidant properties (Citronberg et al., 2013; Kumar et al., 2014; Nile and Park,

2015; Zhang et al., 2016). The antioxidant properties of the ginger are mainly associated with the presence of gingerol, shogaol, oleoresin and paradol while the antimicrobial activities are attributed to quercetin, zingerone, gingerenone-A and shogaol against several human pathogens such as *Escherichia coli, Salmonella typhi* and *Bacillus subtilis* (Azu and Onyeagba, 2007; Park et al., 2008; Rampogu et al., 2008; Manjunatha et al., 2013).

In recent years, the search for unique ingredients with beneficial health promoting effects for new product innovation is popular. Ginger honey is one of those healthy, natural and flavourful products available in the market. Even though previous studies had proposed the therapeutic and nutritional properties of ginger and honey, there was only little research conducted for the evaluation of ginger honey mixture (Ewnetu et al., 2014; Wilczyńska et al., 2017). Since there is limited scientific information available for ginger honey, the purpose of this study was to investigate the sensory attributes, physicochemical properties and bioactivities of honey added with ginger. Hence, the objectives of this research were:

i. To evaluate the sensory attributes and the acceptability of ginger honey.
ii. To measure the physicochemical properties of ginger honey.
iii. To examine the bioactivities of ginger honey.
iv. To compare the sensory characteristics, physicochemical parameters and bioactivities between original honey and ginger honey.

CHAPTER 2

LITERATURE REVIEW

2.1 Honey

Honey is a therapeutic natural product which is produced through the combination of flower nectar, plant secretions or blossoms with invertase and digestive enzymes of honey bees thus it is naturally sweet and popularly consumed (Wang et al., 2019). After the combination, honey bees will deposit the mixture in the wax honeycomb and allow it to mature over time (Almasaudi, 2020). Despite mainly (around 80% of its weight) consisting of sugar such as fructose, glucose, maltose and sucrose, honey is often sought after by consumers for its antioxidant, anti-inflammatory and antibacterial properties due to the presence of vitamins, minerals, amino acids, enzymes and phytochemical compounds (Cianciosi et al., 2018). Moreover, Olas (2020) reported that the chemical composition of honey is influenced by the environmental conditions, seasons, processing method and botanical origin which directly affects their biological activities, viscosity and colour.

Honey has always been widely used worldwide since ancient times by the Sumerians, Chinese, Greeks, Romans, Vedics, ancient Egyptians, Assyrians and Islams as a traditional remedy due to its nutrition and medicinal properties (Eteraf-Oskouei and Najafi, 2012; Cilia et al., 2020; Wang et al., 2021). Nowadays, a huge variety of honey that can be categorised according to their floral and geography source plus other bee products such as propolis and royal

jelly are commonly used as alternative treatment for diseases as well as natural sweeteners and preservative in food products due to their antioxidant and antibacterial bioactivities (Wang et al., 2021). In addition, honey was reported to have health benefits such as antitussive, wound healing and treatment for gastroenteritis (Cohen et al., 2012; Eteraf-Oskouei and Najafi, 2013; Albaridi, 2019).

Resh and Cardé (2009) stated that there are four common species of honeybees that produce honey which includes *Apis florea*, *Apis cerana*, *Apis mellifera* and *Apis dorsata*. *A. cerana*, the Eastern or Asian honey bee (Figure 1.1) is found to be widely used in Malaysia for pollination predominantly in areas of Kuala Lumpur, Penang, Terengganu, Pahang, Perak and Sarawak (iNaturalist, 2021). Wang et al. (2019) added that *A. cerana* honey is typically pricier than *A. mellifera* honey due to its long ripening period, strong resistance to diseases and diverse nectar source which consists of flowers of herbs or traditional medicines.

Figure 1.1: Photograph of *Apis cerana* honeybee (iNaturalist, 2021).

2.1.1 Constituents of Honey

Other than sugars, the constituents that can be found in honey are protein, minerals, vitamins and phytochemicals. A research by Cianciosi et al. (2018) stated that honey contains small amounts of free amino acids like proline, alanine, phenylalanine, tyrosine, leucine, isoleucine and glutamic acid. The enzymes present in honey are acid phosphatase, catalase, glucose oxidase and sucrose diastase (Bogdanov et al., 2008). Moumeh et al. (2020) also reported that honey contains essential minerals such as potassium, selenium, zinc, calcium, copper, sodium, manganese, magnesium and phosphorus and vitamins such as vitamin C and B complex (B1, B2, B3, B5 and B6). Organic acids such as gluconic, tartaric, malic, citric, succinic, acetic, formic, fumaric, oxalic, lactic and many others are present in honey (Mato et al., 2003; Nozal et al., 2003; Tezcan et al., 2011). The main compounds that contributed to antioxidative and antibacterial activities of honey are its phytochemicals especially phenolic acids and flavonoids as summarised in Table 2.1 (Almasaudi, 2020).

Table 2.1: Compounds commonly found in honey and their respective functions.

Compound		Function	Source
Phenolic acid	Syringic acid	-Antimicrobial effect by causing the cell membrane of bacteria to dysfunction	(Griep et al., 2007)
	Chlorogenic acid	-Antibacterial effect by increasing the permeability of cell membrane thus causing cytoplasmic and nucleotide leakage	(Górniak et al., 2019)
	Caffeic acid	-Antibacterial effect by strong nucleophilic properties and damaging the integrity of cell membrane which destroyed membrane function of *Staphylococcus aureus*	(Vaquero et al., 2007; Luís et al, 2014; Ng et al., 2021)

	Gallic acid and ferulic acid	-Antibacterial effect by causing irreversible alteration to bacterial membrane properties which caused essential intracellular constituents to leak	(Borges et al., 2013)
	p-Coumaric acid	-Antibacterial effect by disrupting the structure of cell membrane and binding to the DNA of bacteria	(Borges et al., 2013)
Flavonoids	Kaempferol	-Protective effects against cardiovascular disease (CVD) by suppressing tumour necrosis factor-α production, activating nuclear factor-κB, activating Ca^{2+}-activated K^{+} channels and increasing endothelial nitric oxide synthase activity	(Olas, 2020)
	Chrysin	-Cardioprotective effect -Antioxidative effect, decrease lipid synthesis and increase lipid metabolism -Modulate vascular function by increasing nitric oxide's bioavailability -Anti-inflammatory effect by inhibiting nuclear factor-κB signalling pathway	(Olas, 2020)
	Catechin	-Antibacterial effect by damaging cytoplasmic membrane of *Escherichia coli* -Inhibit blood platelet aggregation when binded to thromboxane A_2 receptor	(Olas, 2020; Ng et al., 2021)
	Apigenin and luteolin	-Inhibit blood platelet aggregation when binded to thromboxane A_2 receptor	(Olas, 2020)
	Quercetin	-Inhibit blood platelet aggregation when bound to thromboxane A_2 receptor -Lower blood pressure while restoring endothelial dysfunction	(Olas, 2020)
	Pinocembrin	-Antioxidative, antimicrobial and anti-inflammatory effect	(Olas, 2020)
	Galangin	-Protective effect against cardiac remodelling by decreasing apoptosis and inflammatory responses	(Olas, 2020)
Gluconic acid		-Antibacterial effect by interfering metabolism of bacteria (except acidophiles)	(Oryan et al., 2016)
Hydrogen peroxide		-Antibacterial effect by producing radicals and interacting with other antibacterial components thus causing DNA damage to bacteria	(Brudzynski and Lannigan, 2012)

2.1.2 Aromatic Components of Honey

Besides the components of honey stated above, Wang et al. (2019) reported that more than 600 volatile compounds have been identified in honey using methods like gas chromatography-mass spectrometry and electric nose as summarised in Table 2.2. Wang et al. (2019) added that the presence of these volatile compounds are the reason why different floral or nectar sourced honeys have their unique aroma. However, at certain temperatures and time of exposure, changes to aroma, colour and concentration of volatile compounds may occur (Manyi-Loh et al., 2011).

Table 2.2: Aromatic components of honey and their respective odour description (Manyi-Loh et al., 2011; Tian et al., 2018).

Component		Odour description
Alcohols	Ethanol	Alcohol
	1-propanol	Alcohol, ripe and fruity
	2-methyl-1-propanol	Solvent and bitter
	Butanol	Medicinal and phenolic
	3-methylbutanol	Fusel, alcohol, sweet and fruity
	Pentanol	Sweet and burning
	Leaf alcohol	Grassy-green
	2-ethylhexanol	Rosy and sweet
	2,3-butanediol	Burnt
	Furfuryl alcohol	Burnt
	2-phenylethanol	Flowery, rose and honey-like
Esters	Ethyl acetate	Pineapple-like
	Ethyl-2-methylbutyrate	Green-fruity and apple-like
	Ethyl 3-methylbutyrate	Fruity
	Ethyl lactate	Fruity

	Ethyl benzoate	Flowery, celery-like and fruity
	γ-valerolactone	Sweet and herbaceous
Aldehydes	Acetaldehyde	Pungent and ether
	3-methyl-butanal	Fruity and fatty
	Hexanal	Grassy, tallow and fatty
	Octanal	Fatty, soapy, lemony and green
	Nonanal	Fatty, greenish floral, citrusy and spiny
	Furfural	Bread-like, soft almond sweet and fruity
	Benzaldehyde	Almond, sweet burnt sugar and marzipan
	5-methyl furfural	Sweet and spicy
	2-phenylacetaldehyde	Hawthorne, honey-like and sweet
	2-phenyl-2-butenal	Green, floral and woody
	Anisaldehyde	Minty and sweet
Ketones	Acetone	Sweet and fruity
	2-pentanone	Ethereal and fruity
	2,3-pentanedione	Sweet
	2-octanone	Floral, bitter, green and fruity
Acids	Acetic acid	Sour and vinegar-like
	Butyric acid	Rancid, cheesy and sweat-like
	Isovaleric acid	Sweat, acidic and rancid
	Hexanoic acid	Sharp, sour, rancid and goat-like odour
	Octanoic acid	Sweat, cheesy
	Nonanoic acid	Green and fatty
	3,7-dimethyl-6-octenoic acid	Green-grassy
	Geranic acid	Sweet and floral
Heterocyclic compounds	Dimethyl disulfide	Onion-like, cabbage-like and putrid
	2-acetylfuran	Coffee-like
Phenols	Carvacrol	Pungent

2.2 Ginger

Ginger (*Zingiber officinale* Roscoe) is a spicy aromatic plant in which its rhizomes are commonly used as functional food and medicine (Indiarto et al., 2020). Indiarto et al. (2020) stated that ginger is a herbaceous plant which possesses a pseudostem where numerous narrow leaves emerge from whereas its noticeable appearance is perennial rhizomes, fibrous roots and annual aerial parts. Ginger consists of carbohydrates (60% - 70%), water (9% - 12%), protein (9%), ash (8%), crude fibre (3% - 8%), lipids (3% - 6%) and volatile as well as less volatile components (1% - 3%) which will determine the flavour and aroma of plant (Habtemariam, 2019; Kim and Kim, 2010). Furthermore, the chemical composition of extracted ginger oil was reported to be influenced by freshness, extraction method, dryness and source of rhizome (Mahboubi, 2019).

According to Indiarto et al. (2020) ginger is classified as Generally Recognized as Safe (GRAS) by Food Drug and Administration (FDA) thus it is utilised in a variety of food, beverages, traditional medicine (Ayurvedic, African, Arabic, Chinese and Japanese), spices and essential oils and they can be used in a many forms such as fresh, dried, powder, essential oil, durable, paste, oleoresin or pickled. Being pungent, aromatic, bioactive and low priced as well as widely available, ginger was found to be used by the Greeks and Romans during 1st AD then Europe during 9th century whereas it became popular gradually in 13th and 14th centuries. In the present, ginger is still a widely used plant worldwide especially in South-east Asia where it was said to be its origin (Habtemariam, 2019). Ghasemzadeh et al. (2010b) stated that since 2000 years ago, ginger has already been used generally in Malaysia for medicinal and food purposes. Based

on a research done by Suhaimi et al. (2014) Malaysian gingers were usually cultivated in Bentong (Pahang), Bakun (Sarawak) and Keningau (Sabah) where the main commercially preferred varieties were Bentong, Bara, Chinese and Indonesian ginger.

As shown in Figure 1.2, Bentong ginger (*Zingiber officinale* Roscoe var. Bentong) has a more superior quality due to its rather different physicochemical content and antioxidant effect compared to other Malaysian gingers (Nafi et al., 2014).

Figure 1.2: Photograph of Bentong ginger (Sinchew, 2020).

According to Zahid et al. (2021), Bentong ginger was reported to have a total phenolic acid content of 16.95 mg GAE/g DW and total flavonoid content of 28.83 mg RE/g DW whereas its antioxidant activity was 66.72% for DPPH assay and 50.90 mg TE/g DW for FRAP assay. In addition, Nafi et al. (2014) added that the enzyme that can be extracted from Bentong ginger's rhizome was

classified as a cysteine protease while having an optimum activity at 60℃ and pH ranging from 6 to 8.

2.2.1 Constituents of Ginger

Generally, the compounds that lead to medicinal and nutritional functions of ginger are their less-volatile components. According to Ghasemzadeh et al. (2010a) ginger was found to have phenolic compounds like gingerol and shogaol plus flavonoids like quercetin, rutin, catechin, epicatechin, naringenin and kaempferol. Nikkhah-Bodaghi et al. (2019) added that ginger which is a potent antioxidant agent could be utilised to improve ulcerative colitis. Khandouzi et al. (2015) reported that gingerol can be converted into shogaol and zingerone to improve fasting blood sugar, reduce haemoglobin A1c, apolipoprotein B, apolipoprotein A-I and malondialdehyde level of patients with type 2 diabetes. Moreover, ginger was reported to have terpene compounds like α-curcumene, α-farnesene, β-bisabolene, β-sesquiphellandrene and zingiberene, and phenolic acids include pyrogallol p-hydroxy benzoic acid, p-coumaric acid and ferulic acid (Tohma et al., 2016; Mao et al., 2019). Terpenes such as D-limonene were reported to be able to prevent division of cancer cells by inducing apoptosis and increase secretion of enzymes in liver that detoxify carcinogens (Babajide et al., 2013). Furthermore, Beristain-Bauza et al. (2019) stated that phenolic compounds in ginger have a synergistic relationship for antimicrobial effect with other compounds like α-farnesene, α- and β-bisabolene, β-sesquiphellandrene, cis-caryophillene and zingiberene while antimicrobial compounds like arcurcumene, citral, geranial and camphene were reported by Munda et al.

(2018). Early studies reported the functions of various bioactive compounds in ginger which was summarised in Table 2.3.

Table 2.3: Bioactive compounds in ginger and their respective functions.

Compound	Function	Source
Catechin	-Anti-cancer effect on postmenopausal and rectal cancer	(Ghasemzadeh et al., 2010a)
Epicatechin	-Reduces lung tumour metastasis	(Rahman et al., 2011)
Naringenin	-Antioxidative and anti-inflammatory effect -Scavenge free radical, promote carbohydrate metabolism and modulate immune system	(Ghasemzadeh et al., 2010b)
Quercetin	-Iron-chelating activity -Inhibits breast cancer cell lines from growing -Anticarcinogenic effect	(Ghasemzadeh et al., 2010a Rahman et al., 2011)
Kaempferol	-Inhibit ovarian cancer cell lines from growing	(Rahman et al., 2011)
Ferulic acid	-Anti-carcinogenic effect on proliferating liver, breast, colon, nervous system and tongue cells	(Tohma et al., 2016)
Rutin and Baicalein	-Iron-chelating activity	(Rahman et al., 2011)
Shogaol	-Strong antioxidant activity -Weakening effect on diabetes neuropathy -Inhibits anticlastogenic and oxidative stress -Anti-inflammatory effect by inhibiting prostaglandin and leukotrienes biosynthesis through inhibiting muscle pain-reducing cyclooxygenase and lipoxygenase	(Haghighi et al., 2005; Mao et al., 2019)
Zingerone	-Antibacterial effect on *Escherichia coli* and *Bacillus subtilis* -Antioxidative effect by stabilising or neutralising free radicals (ROS) that cause muscle damage and pain -Anti-inflammatory effect by inhibiting prostaglandin and leukotrienes biosynthesis	(Haghighi et al., 2005; Mao et al., 2019)

Gingerol and its derivatives (6-gingerol, 8-gingerol and 10-gingerol)	-Blood glucose lowering effect by increasing glucose uptake at insulin responsive adipocytes -Strong antioxidant activity -Anti-inflammatory effect by inhibiting prostaglandin and leukotrienes biosynthesis	(Haghighi et al., 2005; Khandouzi et al., 2015; Mao et al., 2019)
Paradol	-Antioxidative, anti-inflammatory and anticancer effect	(Rahmani et al., 2014)

2.2.2 Aromatic Components of Ginger

Kim et al. (2016) and Habtemariam (2019) reported that aroma and flavour of ginger are determined by its volatile constituents in which [6]-gingerol and shogaols gives it its pungent and spicy-sweet taste. Moreover, the aroma and taste of ginger depends on its geographical location, isolation method and processing temperature as gingerols were reported to be thermally labile (Habtemariam, 2019). In addition, Nishimura (1995) stated that Japanese fresh ginger rhizomes's odour was described as strong citrusy, camphoraceous, floral, musty, green and fatty while freshly harvested Queensland ginger rhizomes was described as citrussy by Connell and Jordan (1971). Meanwhile, raw ginger from China was described as clove-like, smoky, eucalyptus-like, vanilla-like, citrusy and bergamot-like by Schaller and Schieberle (2020a). According to Nishimura (1995) and Schaller and Schieberle (2020a), there are various components had been identified which contribute to the aroma of ginger.

Table 2.4: Aromatic components of ginger and their respective odour description (Nishimura, 1995; Schaller and Schieberle, 2020a).

Component	Odour description
(E)-2-decenal	Green and fatty
(E)-2-octenal	Green, fatty and nutty
(E)-isoeugenol	Smokey and clove-like

1,8-cineole	Camphoraceous and eucalyptus-like
2-heptanol	Herbaceous and coconut-like
2-undecanone	Musty, fruity and sweaty
4-hydroxy-2,5-dimethyl-3(2H)-furanone	Caramel-like
Acetic acid	Pungent
Citronellal	Citrusy and lemon balm-like
Citronellol	Rosy
Fenchol	Earthy and mouldy
Geranial	Citrusy
Geraniol	Floral and rosy
Isoborneol	Earthy and mouldy
Linalool	Floral, citrusy and bergamot-like
Neral	Citrusy and soapy
Octanal	Citrusy
Vanillin	Vanilla-like

2.3 Honey with Ginger

People usually consume ginger honey tea as a warm drink for stomach discomfort whereas the combination of ginger, garlic and honey is one of the conventional asthma and cough remedies of Indians (Duke, 2003). Ewnetu et al. (2014) proposed that the extract of ginger powder with honey can serve as an antibacterial agent as it is more potent instead of using ginger or honey alone. In addition, it was proven that polyphenols (flavonoids and phenolic acids) in honey and ginger were correlated with antibacterial effects (Silici et al., 2010; Brudzynski et al., 2011). Furthermore, both honey and ginger contain tannin thus they can be used together by applying it topically to bruises, sprains and superficial wounds due to their ability in enhancing the healing of wounds and inflamed mucous membrane (Elmarie and Johan, 2001; Usman and Osuji, 2007).

Subsequently, Tahir et al. (2015) stated that ginger and Gelam honey was found to have antitumor and anti-inflammatory properties which in fact could be an effective chemopreventive plus therapeutic strategy to stimulate apoptosis of colon cancer cells. Moreover, the combination of honey, ginger and tumeric was found to be an effective natural treatment to decrease adverse side effects of chemotherapy for intestinal toxicity which was induced by methotrexate (Kutry, 2019).

Furthermore, in a study proposed by Wilczyńska et al. (2017), the authors investigated the influence of addition of spices including cardamom, cinnamon and ginger on sensory quality of honey, and concluded that addition of ginger resulted in alteration of taste and olfactory attributes as well as degree of acceptance. The study showed that the higher the concentration of the ginger added to the base honey, the higher the taste scores which specifically resulted in increased spiciness, bitterness, resin and pungent tastes while decreasing the sweetness of honey. Regarding the degree of acceptance, Wilczyńska et al. (2017) reported that the texture and colour attributes of ginger honey showed lower mean scores than the control honey. However, the increased concentration of the ginger showed a higher degree of acceptance on taste and aroma attributes. Meanwhile, a study of Wilczyńska et al. (2017) showed that the type and concentration of spices added onto multifloral honey had a direct effect on antioxidant activity including DPPH scavenging activity and total phenolic content, where ginger increased total phenolic content and DPPH radical scavenging activity as the concentration of ginger increased. Furthermore, in a study conducted by Wilczyńska et al. (2017), the minimal inhibitory

concentration (MIC) was determined for bacteria of interest which were *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Staphylococcus aureus* using honey with different types of spices (cardamom, cinnamon and ginger) with different concentrations (0.5%, 1% and 2%). The study proved that addition of ginger into honey could inhibit the growth of bacteria. Nonetheless, spiced honey were unable to exhibit greater antimicrobial activity compared to base honey, where honey with and without ginger shared same MIC value of 12.5% w/v for *E. coli*, *P. aeruginosa*, *S. epidermidis*, 6.25% w/v for *S. aureus.* In contrast, a study by Ewnetu et al. (2014), showed the synergistic effect of honey ginger mixture on antimicrobial effects on *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *S. aureus* (MRSA), *E. coli* (R), and *K. pneumoniae* (R). In comparison to the use of honey and ginger extracts individually which found to have average zone of inhibition of 21.63 mm and 19.23 mm, respectively, the mixture of honey and ginger extracts showed the highest average zone of inhibition which was 25.62 mm. Briefly, previous studies had shown that addition of ginger to honey could affect sensory characteristics in terms of visual, taste, olfactory attributes and degree of acceptance of a product, antioxidant capacity and antibacterial properties of ginger honey.

2.4 Sensory Analysis

2.4.1 Honey Evaluation

Sensory analysis is a method to evaluate a product's attributes using human senses which are sight, smell, taste, touch, and sound. Kemp et al. (2009) stated that sensory analysis is useful in comparing the sensory differences between

different products, providing insights for product development, examining the impact of modification to the product ingredients or production process whether will affect sensory characteristics and product acceptability, identifying the main sensory attribute that contribute to acceptability and rectifying the complaints from consumers. To perform sensory analysis, it is simple and low cost, but reproducibility is low (Piana et al., 2004). According to Araujo et al. (2020), geographical origin influences the sensory characteristics of honeys and due to this, the intrinsic quality of different types of honeys are different as well as the degree of acceptance of different honeys by consumers. Hence, it is important to have complementary study of sensory attributes for physicochemical evaluation of honey. Piana et al. (2004) claimed that sensory evaluation is a tool for exploring the botanical origin of honey and determining the imperfections present such as off-aroma and tastes, impurities and fermentation.

2.5 Physicochemical Properties

2.5.1 Introduction

As reported by Council of the European Union (2002), the sensorial, chemical, physical and microbiological characteristics of honey are important in ensuring its quality. Both chemical and physical properties parameters, also known as physicochemical properties, includes pH, colour intensity, water activity, sugar content, moisture content, hydroxymethylfurfural (HMF) content, hydrogen peroxide, proline, diastase and electrical conductivity. On top of that, as reported by previous studies, some of the physicochemical parameters, for example, acidity, sugar content, and water activity are related to antimicrobial properties, colour intensity is linked to antioxidant properties, moisture content driven the

physical properties while electrical conductivity is related to ash content and acidity (Araujo et al., 2020; Moumeh et al., 2020).

2.5.2 Colour Intensity

The colour intensity of the honey can be determined by ABS_{450} value that takes into account the polyphenol constituents and the colour classification is according to the different absorption of light of different wavelengths by honey (Moniruzzaman et al., 2013a). As reported by United States Department of Agriculture (USDA) (1985), the colour of honey can be classified into seven groups ranging from water white to dark amber. Based on Dżugan et al. (2018), polyphenols content is related with honey colour. The greater the colour intensity of honey, the higher the total phenolic content, which in turn indicates greater antioxidant activity. Other than polyphenols, the presence of plant pigments such as carotene, anthocyanins, flavonoids and xanthophylls, as well as mineral salts and amino acids will influence color of honey (Taha et al., 2014). Besides, the colour intensity of honey relies on floral origin, storage conditions, mineral content, ash content and heating (Ahmed et al., 2016).

2.5.3 pH

Based on Sereia et al. (2017), the amount of hydrogen ions contained in a solution is referring to pH. By conducting pH analysis, total acidity of honey can be estimated as the actions of minerals and buffer acids are present. The pH of honey ranges between pH 3.5 to pH 5.5, pH varies due to the diversity of plant source with different pH of nectar, and different amount of several acids and mineral and other ash elements. The natural acidity of honey could be attributed

to the action of glucose oxidase that converts glucose into gluconic acid, in which gluconic acid would determine taste and stability against microbial spoilage of honey (Sereia et al., 2017).

Due to low pH level of honey, honey can be considered as an antimicrobial agent to inhibit microorganism development including *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp. and *Streptococcus pyogenes* (Mandal and Mandal, 2011; Moumeh et al., 2020). Apart from that, the natural acidity of honey is able to activate wound healing by stimulating immune cells such as macrophage to engulf bacteria, improving oxygen take up in wound for high energy demand in tissue repair, reducing protein break down, and increasing fibroblast activities to have constant supply of extracellular matrix precursor for structural integrity of connective tissue (Minden-Birkenmaier and Bowlin, 2018).

2.5.4 Sugar Content

As claimed by Sereia et al. (2017), honey is made up of 95% of sugars which contribute to dry matter of honey. The carbohydrates in honey mainly contributed by reducing sugars which are monosaccharides such as glucose and fructose with the proportions of 30% and 40% respectively. Fructose define the hygroscopicity (Kamal and Klein, 2011) and the sweetness of honey and has the ability to maintain honey in liquid state whereas glucose have the tendency to crystalize the honey as glucose is weak in solubility (Hartel and Shastry, 1991). If there is elevated sugar content in honey, this indicates greater osmotic pressure whereby the microbial growth can be prohibited (Snowdon and Cliver, 1996). In addition, there are 10% of disaccharides which are sucrose and maltose present

in honey (Leonard, 2017) and sucrose represents 2-3% of carbohydrates in honey. However, due to early harvesting of the immature honey which causes incomplete enzymatic conversion of sucrose into reducing sugars (Belay et al., 2013) or adulteration of honey whereby honey added with commercial sugars (Adebiyi et al., 2004), sucrose level might be altered.

2.5.5 Moisture Content

Moisture content defines the amount of water molecule present in the honey, and it is depending on bee species, climate conditions, floral source, handling of the beehive, maturity of beehive and honey's storage conditions (Moniruzzaman et al., 2013a; Araujo et al., 2020). According to Olaitan et al. (2007), honey is hygroscopic in which moisture in the air is easily absorbed by honey causing high moisture content of honey that promotes the fermentation of honey by osmophilic microorganism to ethanol and carbon dioxide, reducing shelf life of honey. Based on Sereia et al. (2017), moisture content is crucial in determining the quality of the honey in terms of stability and fermentation whereby honey should contain low moisture content to prevent crystallisation and inhibit the growth of osmophilic microorganism that is responsible for fermentation. This ensures the shelf life, quality of sensory characteristics and nutritional value of the honey.

2.5.6 Water Activity

Water activity (A_w) is a concept for evaluation of the interaction of water with other food components and the metabolism accessibility for microbes (Sereia et al., 2017). Hence, water activity is commonly used to check the stability of food,

to regulate microorganism processes and to assess the type of microbes present in food (Chirife et al., 2006). Referring to Olaitan et al. (2007), honey is known to have the characteristic of microbiota stability as it has high sugar content indicating low levels of free water which reduces water activity of honey hence preventing the activity of microorganism. As a result, the longer shelf life, preservation and quality can be safeguarded. Meanwhile, according to Chen (2019), the factors that will affect honey's water activity includes geographical and botanical origin, type of honey, state of honey and induced fine granulation.

2.5.7 Electrical Conductivity

In honey, there are ionizable organic acids and mineral salts that are responsible for the conductivity in aqueous solution (Baloš et al., 2018). The electrical conductivity is often used in routine honey quality testing to review the botanical origin, authentication of honey and to examine the relationship between the electrical conductivity with ash content and acidity of the honey. According to Yadata (2014) and Sereia et al. (2017), the greater the ash content and acidity, the greater the electrical conductivity. Moreover, there are studies showing that electrical conductivity could be influenced by the floral origin, storage time, mineral contents and the amount of organic acids and protein (Acquarone et al., 2007; Karabagias et al., 2014).

2.6 Antioxidant Properties

2.6.1 Introduction

As claimed by Phaniendra et al. (2014), reactive oxygen species (ROS) are a type of free radicals generated via endogenous pathway such as endoplasmic

reticulum and mitochondria and exogenous pathway through addition of heavy metal, pollution, tobacco smoke and radiation. The free radicals generated in low amounts would be advantageous in normal body conditions. However, when oxygen toxicity occurs, the reactive free radicals like superoxide anion radical ($O_2^{\bullet-}$) and hydroxyl radical (•OH) could cause harmful effects to humans especially when the antioxidant defence system is insufficient, the ROS will have a tendency to attack protein, lipid, and nucleic acid in the human body (Rodrigo, 2009). The reported pathology conditions that result from oxidative stress are atherosclerosis, diabetes, hypertension, Parkinson's disease, Alzheimer's disease, cataract and a variety of cancers like breast, lung, colorectal and prostate cancers (Phaniendra et al., 2014).

On the other hand, the propagation of reactive oxygen species (ROS) could be catalysed by iron as it will disrupt redox homeostasis as long as it is present in excess within cells and tissues (Spanierman, 2021). According to Lalhminghlui and Jagetia (2018) hydroxyl radicals are highly reactive and have a short lifespan but possess detrimental effects on proteins and nucleic acids. Based on the Haber-Weiss/Fenton reaction, in the presence of iron ions, hydrogen peroxide will generate hydroxyl radicals with high reactivity which are detrimental to the cell and its components followed by the whole organism (Lalhminghlui and Jagetia, 2018). Meanwhile, superoxide radical, $O_2^{\bullet-}$ will be generated during cellular respiration due to the incomplete metabolism of oxygen and are considered less toxic but in the presence of iron they could be converted into highly reactive hydroxyl radicals, •OH (Lalhminghlui and Jagetia, 2018). Subsequently, $O_2^{\bullet-}$ deals damage to biomolecules through the formation of

hydrogen peroxide, H_2O_2, •OH and peroxynitrite or singlet oxygen directly or indirectly (Lalhminghlui and Jagetia, 2018). Therefore, it is crucial to have antioxidant mechanisms to protect from the negative impacts of reactive free radicals.

Honey and ginger exhibit antioxidant activities by having bioactive compounds. As reported by Dżugan et al. (2018) and Mao et al. (2019), honey contains polyphenols which are phenolic acids and flavonoids, Vitamin C and E, enzymes such as catalase and peroxidase, and trace elements while the ginger has bioactive components like phenolic compounds mainly gingerols, shogaols and oleoresin. By having these bioactive components, honey and ginger exhibit antioxidant properties as a result of mechanisms such as decreasing the lipid peroxidation, raising the expression of antioxidant agent including antioxidant enzymes, antioxidant response elements and glutathione as well as sequestering the free radical to reduce the oxidative stress and hence preventing the pathology conditions (Mashhadi et al., 2013; Dżugan et al., 2018; Mao et al., 2019; Nayaka et al., 2020).

2.6.2 Antioxidants Assays

The antioxidant capacities of honey can be evaluated using many analytical techniques (Moniruzzaman et al., 2012). The four antioxidant assays, including measuring the total phenolic content, iron chelating activity, superoxide anion radical scavenging activity and hydroxyl radical scavenging activity were used in this study to evaluate the antioxidant activities of the honey samples.

The principle of the assay to measure total phenolic content is to extract the total phenolics from samples and to measure the phenolics content using a spectrophotometer at the wavelength range of 690 nm to 710 nm (Bioquochem, 2019). It is a colorimetric assay whereby a reference substance, gallic acid, was required as a standard. Upon the reaction of the Folin-Ciocalteu reagent with phenolic compounds, a blue phosphotungstic-phosphomolybdenum complex will be formed. Blainski et al. (2013) added that in order to achieve maximum absorption of this complex, the amount of phenolics and alkaline solution must be considered.

Furthermore, iron chelating activity of a substance can be measured by Ferrous-ferrozine method. Based on Martinello and Mutinelli (2021), in a mixture of ferrozine solution and ferrous sulphate solution, the ferrozine combines with ferrous ions to produce ferrozine-Fe^{2+} violet complexes with strong absorbance at 562 nm. However, if an antioxidant or chelating agent is present, it will disrupt the reaction by binding to ferrous ions which result in lesser free ferrous ions available to complex with ferrozine, hence the complex formation is limited. As a result, there will be a low concentration of ferrozine-Fe^{2+} violet complexes in the reaction, which in turn generates lesser chromophores thus resulting in a decrease of absorbance.

Moreover, Hazra et al. (2008) proposed that the superoxide anion radical scavenging activity is measured by the reduction of nitro blue tetrazolium (NBT) to a purple formazan in the presence of superoxide radicals. When the superoxide radicals are created from dissolved oxygen via the nonenzymatic phenazine

methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) coupling system, the superoxide radicals will reduce the NBT. If an antioxidant agent is present in the sample, the absorbance at 560 nm with samples will decline thus indicating the ability of the test subject to quench superoxide radicals in the reaction mixture.

By using deoxyribose degradation assay, the ability of the samples to inhibit hydroxyl radical-mediated deoxyribose degradation can be examined. The hydroxyl radical obtained from Fenton reaction will attack deoxyribose causing the breakage of cyclic furan ring which then generates malondialdehyde (MDA). When MDA combines with 2-thiobarbituric acid (TBA), a chromogen with α_{max} at 532 nm will be produced. The A_{532nm} value is directly proportional with the amount of hydroxyl radicals present. When there is an antioxidant agent, the absorbance value will decrease, indicating that the radicals were scavenged by the antioxidant as the degradation of deoxyribose was diminished (Li, 2013).

2.7 Antibacterial Properties

2.7.1 Introduction

Traditional methods are being re-evaluated as the society has a demand for natural, organic and cruelty free approach towards remedies for diseases, thus honey and ginger, being natural products famous for their effectiveness, extraordinary yet versatile applications, have been widely used to counter the activity of pathogenic bacteria. Previous studies have discussed several examples of antibacterial activity of honey including *Acinetobacter baumannii*, *Escherichia coli*, *Helicobacter pylori*, *Mycobacterium tuberculosis*,

Proteus spp., *Pseudomonas aeruginosa, Salmonella enterica, Staphylococcus aureus* and *Vibrio cholerae* (Olaitan et al., 2007; Kwakman and Zaat, 2012; Aggad and Guemou, 2014). Earlier studies also showed that ginger has antimicrobial activity against *Bacillus subtilis, Escherichia coli, Mycobacterium tuberculosis, Porphyromonas endodontalis, Porphyromonas gingivalis, Prevotella intermedia and Salmonella typhi* (Hiserodt et al., 1998; Azu and Onyeagba, 2007; Park et al., 2008).

According to Mandal and Mandal (2011), the main antibacterial agent in honey is hydrogen peroxide which is generated from the oxidation of glucose by glucose oxidase in honey (Subrahmanyam, 1996). Due to different concentrations of hydrogen peroxide in honey, the antibacterial effect varies in honey (Molan, 1992). Brudzynski et al. (2012) reported that hydrogen peroxide drive the formation of hydroxyl radicals which generate oxidative stress to macromolecules that interfere with the proliferation of cells and permeability of cell membrane by inducing peroxidation of protein and lipid plus degrading the nucleic acids, as a result, the cell viability will be reduced.

The high osmolarity and low water activity generates an osmotic effect to exert the antibacterial property as there is limited water availability that is required to support the bacterial growth. The osmotic pressure of high sugar content of honey will act on bacterial cell wall causing efflux of water molecules through osmosis. The bacterial cells become flaccid and dehydrated which eventually causes bacteria cells to stop growing in hypertonic sugar solution (Mandal and Mandal, 2011; Albaridi, 2019).

Furthermore, the gluconic acid produced from oxidation of glucose by glucose oxidase would contribute to the acidity of honey which is not preferably for bacterial activity as the optimum pH values for most bacteria to grow range from 6.5 to 7.0 except for acidophiles. Under an acidic environment, the metabolism of most of the bacterias are interfered because the bacterial protein undergoes denaturation and the intracellular ionic contents are altered (Blamire, 2000; Oryan et al., 2016).

In addition, it was proven that polyphenols (flavonoids and phenolic acids) in honey and ginger have been correlated with antibacterial effects (Silici et al., 2010; Brudzynski et al., 2011). For instance, as reported by Nolan et al. (2019), flavonoids (kaempferol and rutin) and phenolic acids (gallic acid and caffeic acid) in honey are found to be associated with antibacterial properties and Rahmani et al. (2014) reported that the more active antibacterial constituents contained in ginger are gingerol and shagelol. In accordance with Nolan et al. (2019), the inhibitory effect of the polyphenols on bacterial growth happens when polyphenols interfere with DNA synthesis by disrupting essential enzymes or complexes that are responsible for DNA synthesis. Additionally, polyphenols also disrupt function and conformation of bacterial membranes by increasing the permeability of membrane towards protons resulting in loss of cytoplasmic components and as a consequence, the integrity of the bacteria cells are not maintained (Kirnpal-Kaur et al., 2011).

2.7.2 Agar Well Diffusion Assay

The antibacterial properties of honey can be evaluated by using agar well diffusion assay, where honey and bacteria have direct contact when honeys are pipetted into well and zone of inhibition represents the inhibitory effect of honey samples towards the tested bacteria (Zainol et al., 2013). Balouiri et al. (2016) added that the strength of inhibition of different honeys can be easily compared on the same plate when the honey diffuses from the well and produces a clear zone when the bacterial growth is inhibited.

2.7.3 *Enterococcus faecalis*

Enterococcus faecalis, a Gram-positive bacterium strive in an environment with a water activity of 0.76 in the normal flora of the intestinal tract, vagina, and less commonly, the oral cavity of healthy individuals and often can be found in soil and water in environment (FAO, 2002; Centers for Disease Control and Prevention (CDC), 2019). According to Public Health Agency of Canada (2016), the host range of this pathogen can be humans, pets and livestock and it can be transmitted through contact with contaminated surfaces, person to person and food. Gastrointestinal tract of humans and animals including insects, mammals, birds and reptiles could be the reservoirs of *E. faecalis* (García-Solache and Rice, 2019). Under normal circumstances, *E. faecalis* will not cause harm to humans. However, if the individual has underlying health conditions or is immunocompromised, the infection risk is high as *E. faecalis* is an opportunistic pathogen. It could cause a wide range of infections including urinary tract infection, periodontitis, endocarditis, bacteraemia, meningitis, and these infections are usually related to healthcare-associated infections (Watson, 2018).

As reported by Carroll et al. (2007), most of the *E. faecalis* strains are susceptible to ampicillin, teicoplanin and vancomycin while resistant to β-lactams, aminoglycosides, tetracyclines, quinolones, chloramphenicol, streptogramins, macrolides and lincosamides. Strains that are not sensitive to vancomycin treatment are called vancomycin-resistant enterococcus (VRE) which causes infection in healthcare settings (CDC, 2019). In brief, the resistance development of *E. faecalis* is through the alteration of peptidoglycan synthesis pathway in which D-Alanine-D-Alanine will be substituted with D-Alanine-D-Lactate or D- Alanine-D-Serine (Ahmed and Baptiste, 2018).

2.7.4 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative bacterium that commonly inhabits the environment (soil, vegetation and water) especially with a water activity of 0.97 and is found on the skin, throats and faeces of healthy persons (Iglewski, 1996; Sandle, 2016). As reported by Pirnay et al. (2009), *P. aeruginosa* is able to infect humans, animals including livestocks, domestic and wild, and plants (flora and fungi). The pathogen can be transmitted via aerosol droplets from infected respiratory tracts, contaminated water or soil, contaminated mechanical respiratory ventilators in hospital settings and the reservoirs for this pathogen are infected humans, animals, contaminated water and soil (Clifton and Peckham, 2010; Public Health Agency of Canada, 2016; Centers for Disease Control and Prevention (CDC), 2019). As stated by Mena and Gerba (2009), *P. aeruginosa* as an opportunistic pathogen that commonly resulting in healthcare-associated infection, it infects patients with high risk who are on ventilators, catheters and after surgery and causes several infections such as pneumonia, urinary tract

infections, surgical site infections and bacteraemia. *P. aeruginosa* exhibits resistance to multiple antibiotics, including β-lactams, aminoglycosides and quinolones (Hancock and Speert, 2000). Although the combination of several antibiotics are frequently used to treat *Pseudomonas* infection, yet the treatment options for multidrug-resistant types of *P. aeruginosa* are limited (CDC, 2019). Generally, there are several resistance mechanisms developed by *P. aeruginosa* which are production of antibiotic-inactivating enzymes, restriction of outer-membrane permeability, expression of efflux systems that pump antibiotics out of the cells, biofilm formation in lungs of infected patients and mutation (Pang et al., 2019).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Honey Samples

In this research, the three tested local honey samples listed in Table 3.1 were manufactured by ECO BEE SHOP SDN BHD in Kulai Johor. The bee species of the honey is *Apis cerana*. All the samples were tightly sealed with parafilm and stored in a dark box at room temperature in order to avoid direct exposure of sunlight as well as crystallisation of raw honey. This aimed to maintain the quality of honey along the experiment (González Lorente et al., 2008).

Table 3.1: Details of honey samples

Symbol	Product	Ingredient
H1	Rainforest wild raw honey	Rainforest wild raw honey
H2	Ginger Honey Bentong (Honey with 10% ginger)	Rainforest wild raw honey, 10% bentong ginger
H3	Ginger Honey Bentong (Honey with 18% ginger)	Rainforest wild raw honey, 18% bentong ginger

3.1.2 Bacterial Samples

The bacteria used in this research were Gram-positive *Enterococcus faecalis* ATCC 29212 and Gram-negative *Pseudomonas aeruginosa* ATCC 27853, which were obtained from the Faculty of Science, UTAR. The *E. faecalis* bacteria was enriched with nutrient broth and cultured with mannitol salt agar and nutrient agar while the *P. aeruginosa* was cultured with MacConkey agar and nutrient agar.

3.1.3 Chemicals and Media

All the chemicals and media used in this research were listed in Table A of Appendix B.

3.1.4 Equipment and Labware

The equipment and labware used in this research were listed in Table A of Appendix C.

3.1.5 Reagents Preparation

The reagents used in this research were listed in Appendix D.

3.2 Methodology

3.2.1 Research Methodology Overview

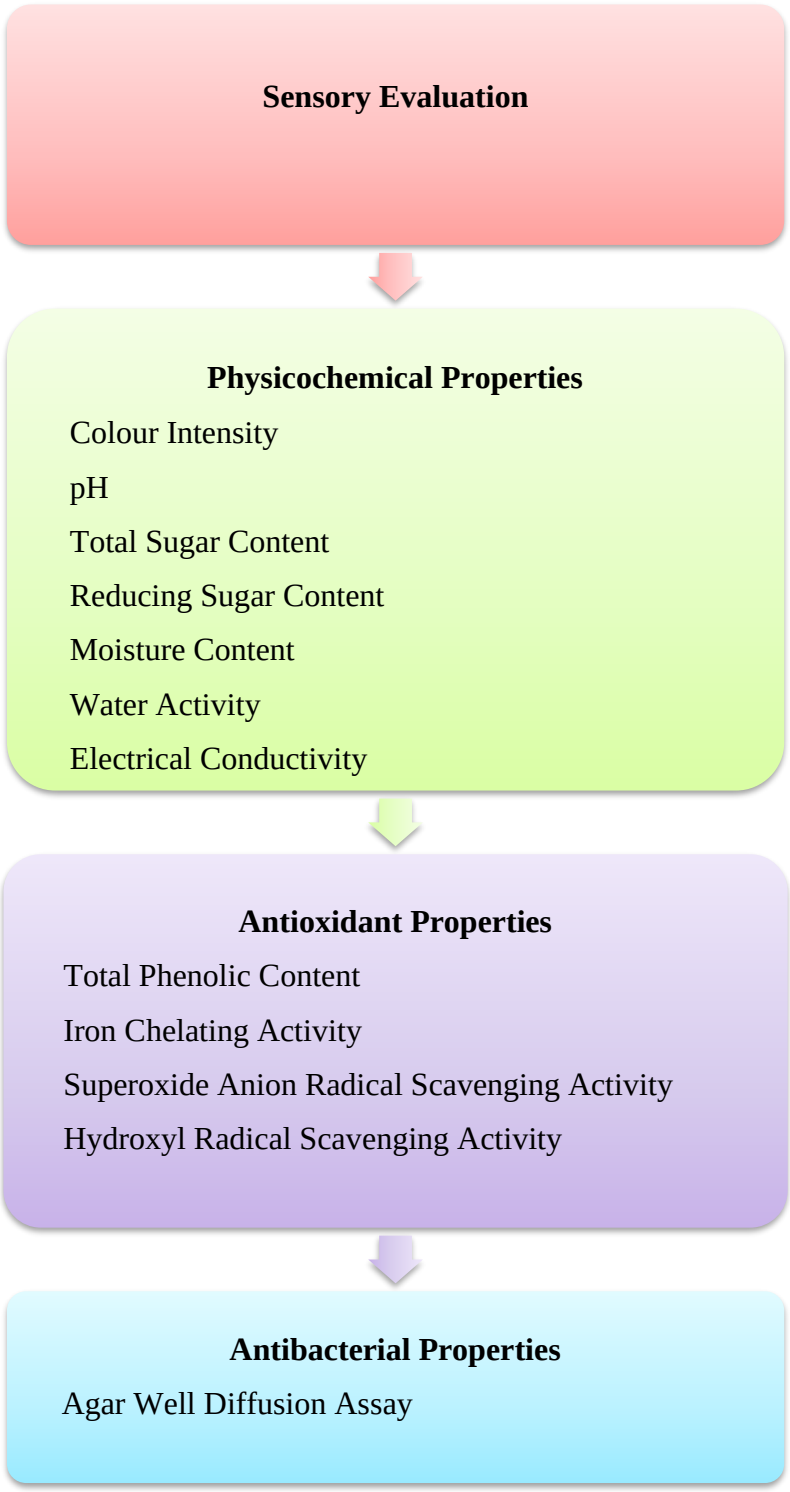

Figure 3.1: Overview of experimental design

3.2.2 Sensory Evaluation

Eight trained panellists included four students, two lecturers and two lab officers from the Department of Agriculture and Food Science, Faculty of Science, UTAR, were invited to evaluate the visual, olfactory and gustatory characteristics and acceptability of honey samples. The guidelines were received by the panellist prior to the sensory evaluation session and the panellists were briefed with instructions to ensure the panellists had clear understanding on the evaluation process. The complete guidelines for the panellists were shown in Appendix F. The sample preparation was carried out on the same day of evaluation to ensure the quality and freshness of the samples. The sterile transparent glass vials were filled with approximately 10 g of honey samples and sugar solution and were tightly capped. The sugar solution was served as a control and was made into a similar colour as the honey samples by adding a drop of edible brown food colouring. The master sheet and score sheets were prepared as shown in Appendix G. On the master sheet, each of the samples were assigned with 3-digit random code and the order of presentation of samples were determined. The assigned random codes were labelled on the bottle and cap of glass vial. On the score sheet, the panel number, date and the random code numbers were filled. The samples were arranged following the order of presentation on a tray. The disposable plastic spoons, coffee bean, water, pencil and score sheet were placed on the tray and tissue paper was prepared for the panellists. Subsequently, the samples were served to the panellists for evaluation. After all the sensory analysis sessions were conducted, the score sheets collected were decoded and data analysis was performed.

3.2.3 Colour Intensity

The colour intensity of honey samples were examined with the method proposed by Moniruzzaman et al. (2013a). In 2 mL of warm distilled water, 1 g of honey sample was dissolved to obtain a 0.5 g/mL of honey solution. The honey solution was then filtered using a 0.45 μm syringe filter. The absorbance of the honey solution was read by using FLUOstar® Omega microplate reader at 450 nm and 750 nm. The measurement of the absorbance was performed in triplicates. The colour intensity was calculated using the formula below and the value was expressed as milli difference (mAU).

Colour intensity = A_{450} - A_{720}

Where

A_{450} = Absorbance at 450 nm;

A_{720} = Absorbance at 720 nm.

3.2.4 pH

By using calibrated Eutech pH 2700 Meter, the pH of honey was measured. Approximately 10 g of honey was dissolved in 75 mL of distilled water to produce a final concentration of 0.13 g/mL of honey solution. A triplicate measurement was performed, and the direct reading was taken from the device followed by calculation of average pH value (Bogdanov, 2009).

3.2.5 Total Sugar Content

The total sugar content of each honey sample was measured by using Atago® pocket refractometer. Prior to the measurement of total sugar content, approximately 80 μL of distilled water was placed on the measuring platform of

the refractometer for calibration. Then, the distilled water was replaced with a honey sample and the refractive index was recorded. The measurement of each honey sample was performed in triplicates and the calculated average value was expressed in mean of percentage, % (g/100g) (Moniruzzaman et al., 2013a).

3.2.6 Reducing Sugar Content

By adapting the method proposed by Moniruzzaman et al. (2013b), the reducing sugar content of honey was determined. Approximately 1 g of honey sample was dissolved in 10 mL of distilled water to obtain 0.1 g/mL of honey solution. Further 100-fold dilution of honey solution was performed to produce a final concentration of 0.001 g/ml honey solution by mixing 0.1 mL of honey solution with 9.9 mL of distilled water. Subsequently, a series of glucose standard solutions with different concentrations were prepared. Approximately 3 mL of honey solution, glucose standard solutions and distilled water (as blank) were mixed with 3 mL of DNSA solution followed by incubation in a boiling water bath for 10 minutes. The solutions were added with 1 mL of Rochelle salt after the solutions were cooled to room temperature. The absorbance of all the sample solutions and blanks were measured in triplicates at 540 nm with FLUOstar® Omega microplate reader. After a standard curve of absorbance against concentration of glucose standard solution was plotted, the reducing sugar content of each sample was calculated and expressed as percentage (%) by using the following equation:

$$R = C / D \times 100$$

Where

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

C = Corrected concentration of glucose with dilution factor (µg/mL);

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

3.2.7 Moisture Content

By using the results obtained from measurement of total sugar content, the estimation of moisture content percentage for each honey sample was calculated with the following formula (Association of Official Analytical Chemists, 1990):

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

3.2.8 Water Activity

The water activity of honey was measured using Novasina Lab Swift portable water activity meter at room temperature. The device was calibrated by using salt standards prior to measurement. The honey sample was filled into a capsule until the indicator line and the capsule was placed on the measuring chamber. The cover of the device was closed and waited for the vapour balance. A direct reading was taken from the display. The measurement was triplicated for all the honey samples followed by calculation of average water activity value (Sereia et al., 2017).

3.2.9 Electrical Conductivity

Approximately 6 g of honey sample was dissolved in 30 mL of distilled water to produce a 0.2 g/mL of honey solution. The electrical conductivity of honey was measured by using the OAKTON Multi-Parameter PCSTestr™ 35 (International Honey Commission, 2009). The measurement was performed thrice to obtain an

average value and the value was expressed in microSiemens per centimetre (μS/cm).

3.2.10 Total Phenolic Content

The Folin-Ciocalteu assay, which was modified by Khalil et al., (2012) were adopted to determine the total phenolic content of the honey samples. As shown in Table A of Appendix E, a series of gallic acid standard solutions were prepared accordingly. The honey solution with a concentration of 0.2 g/mL was prepared by adding approximately 1 g of honey sample into 5 mL of distilled water. Into 0.5 mL honey solution, 0.5 mL of distilled water (as blank) and 0.5 mL of different concentration of gallic acid standard solution, 0.5 mL of Folin and Ciocalteu's phenol reagent were added and left for 3 minutes. Subsequently, 0.5 mL of 10% (w/v) Na_2CO_3 solution was added to the mixture and the solution was topped up with 3.5 mL of distilled water to reach a final volume of 5 mL. The reaction mixtures were incubated in the dark at room temperature for 90 minutes. The absorbance of each reaction mixture was read in triplicates at 725 nm with FLUOstar® Omega microplate reader. Then, the calculation of the total phenolic content of the samples were performed by using following equation:

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

Where

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

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

V = Volume of honey sample used (mL);

M = Mass of honey sample used (kg).

3.2.11 Iron Chelating Activity

As proposed by Chai et al. (2014), the iron chelating activity of the honey samples were determined by ferrous-ferrozine complex assay. A concentration of 0.2 g/mL of honey solution was prepared by dissolving 1 g of honey into 5 mL of distilled water. Into 0.2 mL of the honey samples and distilled water as control, 0.2 mL of 1 mM ferrous sulphate solution and 0.4 mL of 0.25 mM ferrozine solution were added. The honey samples were filtered by using a 0.45 µm nylon syringe filter after incubation of 10 minutes at room temperature. The absorbance of the samples and blank solution were measured in triplicates at 562 nm by using FLUOstar® Omega microplate reader. Subsequently, the iron chelating activity of the honey samples were calculated using the following formula:

$$\text{Iron chelating activity (\%)} = 1 - (A_1/A_0) \times 100$$

Where

A_0 = absorbance of the control;

A_1 = absorbance of the sample.

3.2.12 Superoxide Anion Radical Scavenging Activity

The superoxide anion scavenging activity of the honey samples were measured based on the method reported by Robak and Gryglewski (1988). The honey solutions with 0.2 g/mL concentration were prepared by mixing approximately 1 g of honey into 5 mL of distilled water. Thereafter, 3 mL of 100 mM sodium phosphate buffer, 1 mL of 150 µM NBT, 1 mL of 468 µM NADH, 1 mL of honey solution and 1 mL of 60 µM PMS were mixed. The reaction mixtures were incubated in dark at room temperature for 5 minutes. The absorbance of

the mixtures were measured at 560 nm by using FLUOstar® Omega microplate reader. The percentage of superoxide radical scavenging of each sample was calculated according to the following formula:

Superoxide radical scavenging activity (%) =1 - (A_1/A_0) x 100

Where

A_0 = absorbance of the control;

A_1 = absorbance of the sample.

3.2.13 Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of the honey sample was examined using the deoxyribose degradation method recommended by Li (2013). The 0.2 g/mL honey solution was prepared by adding approximately 1 g of honey into 5 mL of distilled water. Next, 50 µL of honey solution and distilled water as control were added with 400 µL of 0.2 M phosphate buffer, 50µL of 50 mM deoxyribose, 50 µL of 1 mM Na_2EDTA, 50 µL of 3.2 mM $FeCl_3$, 50 µL of 50 mM H_2O_2 and 50 µL of 1.8 mM ascorbic acid. Then the reaction mixture was adjusted to a final volume of 800 µL with the buffer solution. After 20 minutes of incubation at 50°C, a volume of 250 µL of 10% (w/w) TCA and 150 µL of TBA (5%, in 1.25% NaOH aqueous solution) were added into the mixture. Then, the mixture was incubated at 105°C for 15 minutes. The mixture was cooled, and the absorbance was measured in triplicates at 530 nm by using FLUOstar® Omega microplate reader. The average value of hydroxyl radical scavenging activity of the honey was calculated and reported as the percentage of inhibition of deoxyribose degradation according to the following equation:

% inhibition =1 - (A_1/A_0) x 100

Where

A_0 = absorbance of the control;

A_1 = absorbance of the sample.

3.2.14 Antibacterial Properties

The antibacterial activity of the honey samples were observed by adopting a modified agar well diffusion method proposed by Mohapatra et al. (2011). Muller-Hinton agar plates were prepared by pouring approximately 25 mL of autoclaved Muller-Hinton agar on a sterile petri dish. The sugar solution with similar sugar content of honey and 10 μg/mL of ampicillin solution were prepared and autoclaved as listed in Appendix D. The 0.5 McFarland bacterial suspensions of *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853 were prepared by inoculating the fresh bacteria colonies of both bacterial cultures into the tubes filled with 15 mL of sterile 0.85% saline solution until the turbidity of the bacterial suspensions achieved an optical density reading of 0.08-0.13 at 625 nm wavelength, which also equivalent to 1×10^8 CFU/mL (Andrews, 2009). Thereafter, the tip of the sterile cotton swab was soaked into the bacterial suspension and pressed against the tube wall with firm pressure to remove excess fluid. The cotton swab with bacteria was streaked evenly on the surface of the agar entirely with different angles. The four wells on the agar plates for honey samples and sugar solution and two wells on the control agar plates were made by using a sterile cork borer with 6 mm diameter. Approximately 90 μL of the three honey samples, sugar solution, distilled water (negative control) and ampicillin solution (positive control) were filled into wells accordingly. The agar plates were then sealed

tightly with parafilm and kept in an incubator for overnight incubation at 37°C. After that, the diameter of the zone of inhibition for each honey sample was measured to the nearest centimetre (cm). The agar plates for the assay were prepared in triplicates in order to obtain the average value of zone of inhibition for all the honey samples.

3.2.15 Statistical Analysis

All the measurements for each assay were performed in triplicates to obtain the mean and standard deviation for the results. The results were subjected to ANOVA and Tukey's HSD multiple comparison test by using IBM SPSS Statistics software version 28.0.1.0 in order to evaluate the differences of mean values among the honey samples. A right -tailed value of $p < 0.05$ was considered statistically significant.

CHAPTER 4

RESULTS

4.1 Sensory Evaluation

Based on the schematic representation of the visual and texture attributes in Figure 4.1, the colour intensity of the sugar solution, original honey, honey with 10% ginger and honey with 18% ginger displayed slight differences where the original honey had the lowest colour intensity which was 50.0%, followed by sugar solution and honey with 10% ginger, 57.5% and 62.5%, respectively and honey with 18% ginger had the darkest colour which was 67.5%. In contrast, there were huge differences in their viscosity and adhesiveness in which the honey with 10% ginger had the highest viscosity, 92.5% and adhesiveness, 87.5% while sugar solution had the lowest viscosity, 22.5% and adhesiveness, 20% among the four samples. The viscosity and adhesiveness of the original honey were 70% and 72.5%, respectively, while the honey with 18% ginger was found to have 67.5% of viscosity and 75% of adhesiveness.

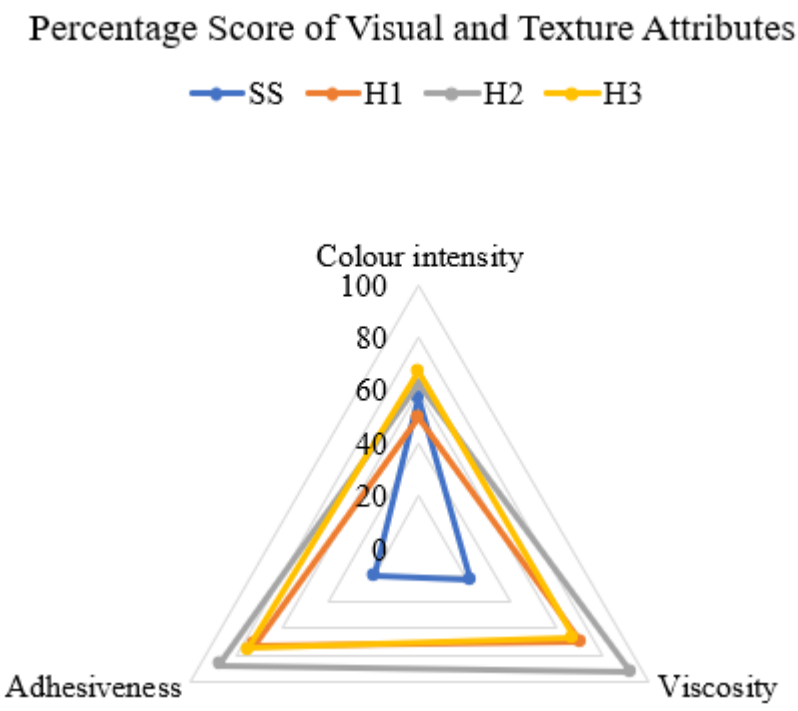

Figure 4.1: Schematic representation of percentage score of visual and texture attributes for sugar solution (SS), original honey (H1), honey with 10% ginger (H2) and honey with 18% ginger (H3).

For olfactory attribute evaluation, by referring to the schematic representation in Figure 4.2, sugar solution showed low values on chemical, warm and vegetal aroma which were 12.5%, 12.5% and 25.0%, correspondingly, without woody, fresh, floral fresh fruit, and spoiled aroma and this indicated that the sugar solution had the weakest aroma intensity as compared to honey samples and this showed high discrimination power to differentiate sugar solution from the honey because the honey's aroma intensities were higher and varied in which obvious differences were found on woody, fresh, floral fresh fruit and warm aromas among the honey samples. By comparing the original honey, honey with 10% ginger and honey with 18% ginger, honey with ginger had higher similar values in the case of woody aroma (62.5%) and fresh aroma (75.0%), original honey and honey with 10% ginger shared same value for floral fresh fruit aroma which was 75.0% and was higher than honey with 18% ginger which was 50.0%. In addition, the intensity of the warm attribute decreased from 62.5% to 37.5% as the ginger concentration in the honey increased. The spoiled aroma was not detected in all the honey samples. This indicated that the addition of ginger in honey increased the woody and fresh aromas, as well as decreased the warm and floral fresh fruit aromas of honeys.

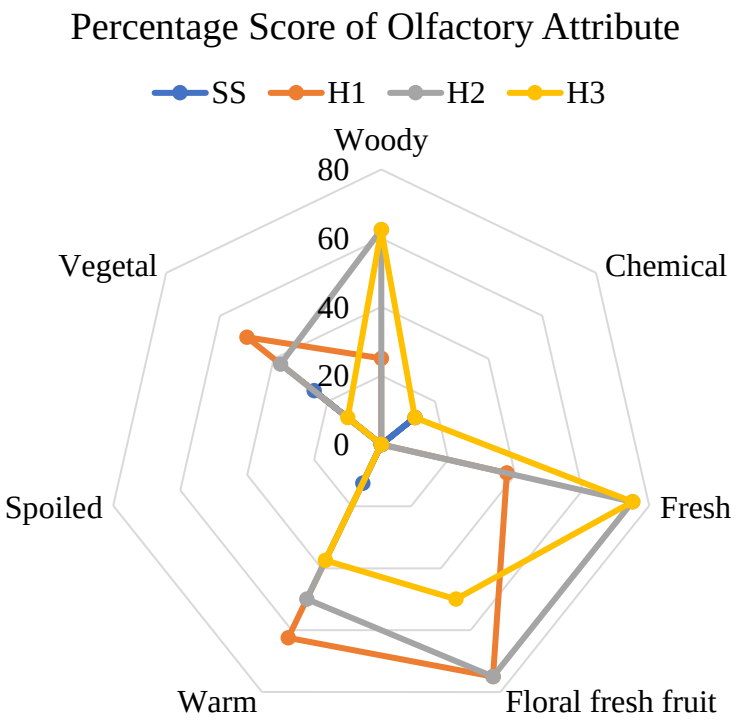

Figure 4.2: Schematic representation of percentage score of olfactory attribute for sugar solution (SS), original honey (H1), honey with 10% ginger (H2) and honey with 18% ginger (H3).

According to schematic representation of the taste attribute of the samples in Figure 4.3, sugar solution showed higher value on sweetness (55.0%), floral fresh fruit and warm (50%) and chemical (37.5%) tastes, while the woody and spoiled tastes were not detected. There were huge differences in the case of woody, fresh, floral fresh fruit, vegetal, astringency, refreshing, sweetness, bitterness, persistence and after taste attributes among the honey samples. In the comparison between the original honey and honey with ginger, ginger honeys had higher woody (62.5%), fresh (50.0 to 70.0%), vegetal (50.0%), astringency (75.0%), refreshing (87.5-100.0%), sourness (35%), bitterness (40.0-42.5%), persistence (75.0-82.5%) and after-taste (77.5-87.5%) values while original honey had greater taste in terms of floral fresh fruit (75.0%), warm (62.5%) and sweetness (80%). No chemical taste was detected among all the honey samples. Generally, the sugar solution had lower score on the taste attributes as shown in olfactory score, the addition of ginger into honey had great impacts on the taste score in which the increased ginger concentration in honey resulted in increased woody, fresh, astringency, refreshing, sourness, bitterness, persistence and aftertaste of the honey whereas the tastes scores of floral fresh fruit, warm and sweetness had declined.

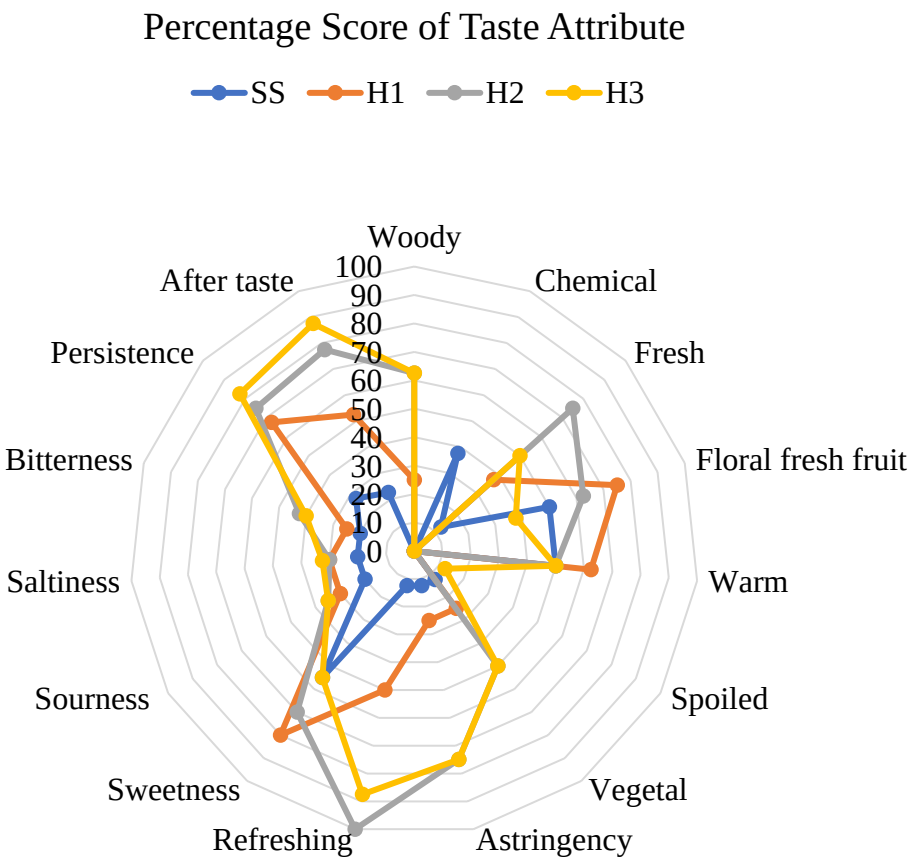

Figure 4.3: Schematic representation of percentage score of taste attribute for sugar solution (SS), original honey (H1), honey with 10% ginger (H2) and honey with 18% ginger (H3).

Lastly, based on the schematic representation of the acceptability score in Figure 4.4, the acceptability among the sugar solution, original honey, honey with 10% ginger and honey with 18% ginger were examined. The degree of acceptability of the samples that were arranged in increasing order was sugar solution, honey with 18% ginger, honey with 10% ginger followed by original honey. This result showed that the addition of ginger into honey reduced the acceptability of the honey as compared to original honey.

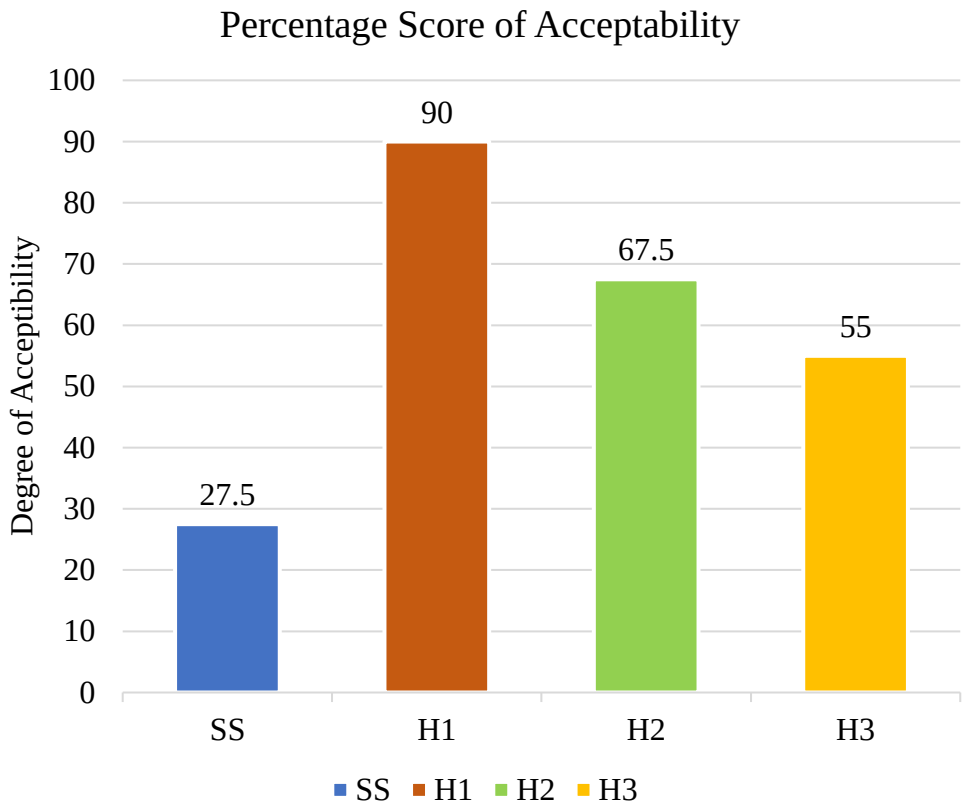

Figure 4.4: Schematic representation of percentage score of degree of acceptability for sugar solution (SS), original honey (H1), honey with 10% ginger (H2) and honey with 18% ginger (H3).

4.2 Physicochemical Properties

Referring to Table 4.1, the colour intensity of the three honey samples ranged from 207.67 mAU to 289.00 mAU, the honey samples processed with addition of 10% ginger and 18% ginger had significantly higher ($p < 0.05$) colour values which are 289.00 mAU and 275.00 mAU, respectively as compared to the original honey which had the lowest colour intensity of 207.67 mAU. The addition of ginger increased the colour intensity of honey, however, the results showed that the increased ginger concentration from 10% to 18%, decreased the colour intensity of honey. With respect to the pH, as shown in Table 4.1, the pH of the three honey samples varied at between 4.58 and 4.85. The pH value of the honey with 10% ginger (4.84 ± 0.01) and honey with 18% ginger (4.85 ± 0.02) had no significant difference ($p > 0.05$) shown whereas the original honey (4.58 ± 0.01) was significantly different ($p < 0.05$) from the two ginger honeys. The honey with 18% ginger had the highest pH value followed by honey with 10% ginger and the lowest was original honey which indicated original honey was the most acidic.

Table 4.1: The mean value for colour intensity and pH of original honey (H1), honey with 10% ginger (H2) and honey with 18% ginger (H3).

Honey samples	**H1**	**H2**	**H3**
Colour intensity (mAU)	207.67 ± 1.53^{a}	289.00 ± 1.00^{c}	275.00 ± 3.00^{b}
pH	4.58 ± 0.01^{a}	4.84 ± 0.01	4.85 ± 0.02^{b}

The measurements were performed in triplicates and the average values were expressed in mean ± standard deviation.
a indicated significant difference between H1 and H2 with $p < 0.05$.
b indicated significant difference between H1 and H3 with $p < 0.05$.
c indicated significant difference between H2 and H3 with $p < 0.05$.

Based on Table 4.2, the total sugar content of the tested honey samples ranged from 78.70% to 81.30% in which the honey with 18% ginger had the lowest total sugar content whereas the honey with 10% ginger had the highest total sugar content which are 78.70% and 81.30%, respectively, and the total sugar content of original honey was 80.10%. In addition, a significant difference ($p < 0.05$) was found among the reducing sugar content of the original honey, honey with 10% ginger and honey with 18% ginger in which the original honey (59.53 ± 0.30%) contained the least reducing sugar content, followed by honey with 10% ginger (60.63 ± 0.67%) while the honey with 18% ginger (63.27 ± 0.21%) contained the most reducing sugar content as summarised in Table 4.2. Furthermore, according to Table 4.2, the estimated moisture content from the total sugar content of the tested honey sample ranged from 18.70% to 21.30%. The original honey and honey with 10% ginger were 19.90% and 18.70%, respectively, while the moisture content of honey with 18% ginger was the highest, which was 21.30%. As stated in Table 4.2, there were no significant differences ($p > 0.05$) shown on water activity between the original honey and honey with 10% ginger in which the water activity of both honey samples were 0.54 whereas the water activity of honey with 18% ginger was 0.55 which was significantly higher ($p < 0.05$) than the former honeys.

Table 4.2: The mean value for total sugar content, reducing sugar content, moisture content and water activity of original honey (H1), honey with 10% ginger (H2) and honey with 18% ginger (H3).

Honey samples	H1	H2	H3
Total sugar content (%)	80.10 ± 0.00	81.30 ± 0.00	78.70 ± 0.00
Reducing sugar content (%)	59.53 ± 0.30[a]	60.63 ± 0.67[c]	63.27 ± 0.21[b]
Moisture content (%)	19.90 ± 0.00	18.70 ± 0.00	21.30 ± 0.00
Water activity	0.54 ± 0.00	0.54 ± 0.00[c]	0.55 ± 0.00

The measurements were performed in triplicates and the average values were expressed in mean ± standard deviation.
a indicated significant difference between H1 and H2 with $p < 0.05$.
b indicated significant difference between H1 and H3 with $p < 0.05$.
c indicated significant difference between H2 and H3 with $p < 0.05$.

Moreover, the electrical conductivity of the three honey samples ranged from 290.33 μS/cm to 535.67 μS/cm. Based on Table 4.3, the significant difference was found ($p < 0.05$) among the electrical conductivity of original honey, honey with 10% ginger and honey with 18% ginger, in which the original honey had the lowest value (290.33 ± 1.53 μS/cm), followed by honey with 10% ginger (446 ± 5.57 μS/cm) and honey with 18% ginger had the highest value (535.67 ± 3.22 μS/cm). As a whole, the addition of 10% and 18% of ginger into original honey had resulted in significant changes ($p < 0.05$) on several physicochemical parameters including colour intensity, electrical conductivity and reducing sugar content which representing the greater discrimination capability between the three honey samples as compared to other physicochemical properties which were water activity, total sugar content, moisture content and pH.

Table 4.3: The mean value for electrical conductivity of original honey (H1), honey with 10% ginger (H2) and honey with 18% ginger (H3).

Honey samples	H1	H2	H3
Electrical conductivity (µS/cm)	290.33 ± 1.53^{a}	446.00 ± 5.57^{c}	535.67 ± 3.22^{b}

The measurements were performed in triplicates and the average values were expressed in mean ± standard deviation.
a indicated significant difference between H1 and H2 with $p < 0.05$.
b indicated significant difference between H1 and H3 with $p < 0.05$.
c indicated significant difference between H2 and H3 with $p < 0.05$.

4.3 Antioxidant Properties

The antioxidant ability of the tested honey samples were evaluated based on the results generated from four assays conducted. As shown in Table 4.4, the total phenolic content of the original honey, honey with 10% ginger and honey with 18% ginger shown significant different ($p < 0.05$) to each other in which the original honey contained the least amount of total phenolic content which was 16.48 ± 0.25 mg GAE/kg, honey with 10% ginger had significantly higher total phenolic content which was 34.62 ± 1.90 mg GAE/kg, whereas honey with 18% ginger had the highest total phenolic content which was 42.52 ± 0.75 mg GAE/kg. Moreover, based on Table 4.4, the iron chelating activity of original honey was shown to be significantly different ($p < 0.05$) from honey with 10% ginger and honey with 18% ginger. However, no significant difference ($p > 0.05$) was found on the iron chelating activity between the honey with 10% ginger and honey with 18% ginger. The iron chelating activity of the original honey (5.45 ± 1.50%) was the lowest followed by honey with 10% ginger (24.61 ± 1.16%) and honey with 18% ginger (25.47 ± 1.14%) which had the highest iron chelating ability. In addition, the significant difference ($p < 0.05$) was shown in the superoxide anion scavenging activity among the original honey, honey with 10%

ginger and honey with 18% ginger in which the original honey had the lowest scavenging value, followed by honey with 10% ginger and honey with 18% ginger had the highest scavenging value which were 56.97 ± 0.30%, 84.27 ± 1.36% and 86.84 ± 0.17%, respectively as summarised in Table 4.4. Besides, referring to Table 4.4, the hydroxyl radical scavenging activity of honey with 18% ginger was found to be the highest which was 72.88 ± 0.43% as compared to the original honey and honey with 10% ginger which had 70.24 ± 0.12% and 70.53 ± 0.09% of the hydroxyl radical scavenging activity, correspondingly. On top of that, the original honey and honey with 10% ginger were found to not significantly differ from each other ($p > 0.05$). Briefly, the addition of ginger into honey had significant contribution ($p < 0.05$) on the increment of antioxidant capacities in terms of total phenolic content, iron chelating activity, superoxide anion scavenging activity and hydroxyl radical scavenging activity especially the honey with 18% ginger had shown to had the greatest antioxidant activities among the three tested honey samples.

Table 4.4: The mean value for total phenolic content, iron chelating activity, superoxide anion scavenging activity and hydroxyl radical scavenging activity assays of original honey (H1), honey with 10% ginger (H2) and honey with 18% ginger (H3).

Honey samples	H1	H2	H3
Total phenolic content (mg GAE/kg)	16.48 ± 0.25^{a}	34.62 ± 1.90^{c}	42.52 ± 0.75^{b}
Iron chelating activity (%)	5.45 ± 1.50^{a}	24.61 ± 1.16	25.47 ± 1.14^{b}
Superoxide anion scavenging activity (%)	56.97 ± 0.30^{a}	84.27 ± 1.36^{c}	86.84 ± 0.17^{b}
Hydroxyl radical scavenging activity (%)	70.24 ± 0.12	70.53 ± 0.09^{c}	72.88 ± 0.43^{b}

The measurement was performed in triplicates and the average value was expressed in mean ± standard deviation.
a indicated significant difference between H1 and H2 with $p < 0.05$.
b indicated significant difference between H1 and H3 with $p < 0.05$.
c indicated significant difference between H2 and H3 with $p < 0.05$.

4.4 Antibacterial Properties

In Table 4.5, the zone of inhibition of *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853 subjected to the sugar solution, original honey, honey with 10% ginger and honey with 18% ginger were displayed. With regards to *E. faecalis*, there was no zone of inhibition observed at the wells of sugar solution and original honey which significantly differ ($p < 0.05$) from the honey added with ginger. There was no significant difference ($p > 0.05$) found in the zone of inhibition between honey with 10% ginger (0.83 ± 0.09 cm) and honey with 18% ginger (0.75 ± 0.09 cm). On the other hand, sugar solution showed no inhibition on *P. aeruginosa* which was similar to *E. faecalis.* Nonetheless, the zone of inhibition among the original honey (0.92 ± 0.09 cm), honey with 10% ginger (0.93 ± 0.04 cm) and honey with 18% ginger (0.96 ± 0.01 cm) were found to be no significant difference ($p > 0.05$) on *P. aeruginosa.*

In brief, the original honey was unable to exhibit inhibitory effect against *E. faecalis* while the addition of ginger into honey was more effective in inhibiting the growth of *E. faecalis*. Moreover, the addition of ginger had no significant impact on the antibacterial effect of honey on *P. aeruginosa*.

Table 4.5: The mean value for zone of inhibition of agar well diffusion assay of sugar solution, original honey (H1), honey with 10% ginger (H2) and honey with 18% ginger (H3).

Honey samples	**Zone of inhibition (cm)**	
	***E. faecalis* ATCC 29212**	***P. aeruginosa* ATCC 27853**
SS	NIL	NIL
H1	0.00 ± 0.00^{a}	0.92 ± 0.09
H2	0.83 ± 0.09	0.93 ± 0.04
H3	0.75 ± 0.09^{b}	0.96 ± 0.01

The measurement was performed in triplicates and the average value was expressed in mean ± standard deviation.
a indicated significant difference between H1 and H2 with $p < 0.05$.
b indicated significant difference between H1 and H3 with $p < 0.05$.
NIL=No zone of inhibition

CHAPTER 5

DISCUSSION

5.1 Sensory Evaluation

5.1.1 Visual and Texture Attributes

Honey colour has a wide range from water white to amber to dark amber and it is based on floral origin, ash content, mineral contents and phenolic content (Lazaridou et al., 2004; Bertoncelj et al., 2007). According to Dżugan et al. (2018), the colour intensity is reflecting the total phenolics content and antioxidant activity of honey. On top of that, similar trend was observed in this study where the colour of ginger honeys were darker than original honey, and as shown in Table 4.4, the total phenolics content of ginger honeys were higher than original honey, and this indicated that the ginger honeys had higher antioxidant activities than original honey. In addition, the darker colour of ginger honey could be due to the higher ash content which was indicated by higher electrical conductivity in ginger honey as shown in Table 4.3 in this study and the data obtained was comparable to studies by Al et al. (2009) and Moumeh et al. (2020) which reported that light-coloured honeys had low ash contents, while dark-coloured honeys had higher ash contents. This showed that the colour intensity of honey is directly proportional to the level of ash content and total phenolics content and the addition of ginger enhanced the colour intensity of honey.

According to Lazaridou et al. (2004) and Yanniotis et al. (2006), the viscosity and adhesiveness of honey are affected by moisture content of honey that depends on the total sugar content of honey, temperature and the crystals or colloids substances in honey. In the respect of moisture content, as shown in Table 4.2, the honey with 10% ginger had the highest total sugar content and the lowest moisture content, and this explained the highest viscosity and adhesiveness scores for honey with 10% ginger which displayed in Figure 4.1. Similarly, a study conducted by Moumeh et al. (2020) showed that the moisture content is important in determining the physical properties of honey whereby the high sugar content in honey causes the honey to have low moisture content and thus makes the honey viscous. Other than that, honey is considered a supersaturated solution which contributes to the viscosity and adhesiveness of honey (Amariei et al., 2020). The supersaturated characteristic of honey is resulted from fructose and glucose content in honey which were 30% - 44% and 25% - 40%, respectively (Amariei et al., 2020). According to Brudzynski et al. (2017), the supersaturated concentration of honey also served as crowding molecules of the honey macromolecules which resulted in colloid particles formation in honey. The colloid substances would affect the degree of viscosity and adhesiveness of honey, and this explained the viscous and adhesive characteristics of honey in this study (Yanniotis et al., 2006). Upon ginger addition process, the physical forces underlying the intermolecular interactions would be affected due to the destabilising of water molecules and the reduced sugar concentration by ginger. This explained the reduced viscosity and adhesiveness of honey by higher concentrations of ginger which was 18%. This proved that the viscosity and adhesiveness of honey were altered by ginger.

5.1.2 Olfactory and Taste Attributes

According to Figure 4.2 and Figure 4.3, the addition of ginger resulted in the increase of olfactory attributes including woody and fresh, and the decrease of floral fresh fruit and warm aromas. The similar trend was observed in taste attributes where the addition of gingers increased the woody and fresh tastes and decreased the floral fresh fruit and warm taste. Moreover, the increased astringency and bitterness, and diminished sweetness, persistence and aftertaste were attributed to the addition of ginger as well.

In honey, furfural, benzaldehyde, 2-acetylfuran, carvacrol are the compounds that were reported to have woody aroma contributions including the pungent, burnt sugar and coffee-like aromas (Tian et al., 2018). The woody aroma increased when ginger was added into honey due to the presence of 6-gingerol, capsaicin, 6-shogaol, (E)-2-octenal, caryophyllene, 3-methyl-2-butene-1-thiol, β-myrcene, (E)-isoeugenol and zingerone from ginger (Babajide et al., 2013; Kim et al., 2016; Schaller and Schieberle, 2020a). Hence, the higher the concentration of ginger in honey, the higher the pungent phytochemicals and odorants of ginger that contributed to the woody aroma of honeys. Besides, based on Tian et al. (2018), there were two compounds, octanal and anisaldehyde, reported to have fresh aroma in honey. When the ginger added into honey, the odorants of ginger such as 1,8-cineol, octanal, 2-undecanone, neral and geranial give citrus- and eucalyptus-like fruity odour which contributed to the increased fresh aroma of ginger honey (Schaller and Schieberle, 2020a). The amount of compounds that had woody and fresh aromas

in honey were lesser than ginger and this explained the lowest woody aroma and freshness of original honey as compared to ginger honey as shown in Figure 4.3.

In contrast, the floral fresh fruit flavour was decreased in ginger honey. The higher floral fresh fruit flavour in original honey was attributed to the compounds in honey including 3-methylbutanol, 1-propanol, 2-ethylhexanol, 2-phenylethanol, ethyl benzoate, 2-phenyl-2-butenal, acetone and geranic acid (Tian et al., 2018). Though ginger had floral fresh fruit components which are 2-heptanol and geraniol, the presence of spoiled taste in ginger which demonstrated by more compounds such as acetic acid, fenchol and isoborneol outweighed the floral fresh fruit components of the honey (Schaller and Schieberle, 2020a) which resulted in decreased floral fresh fruit flavour of ginger honey.

Furthermore, as shown in Figure 4.3, the original honey had the highest warm attribute percentage score as the original honey had the sweetest flavour and it was comparable to the study by Moumeh et al. (2020) which proposed that the sweet flavour was associated with the warm attribute of honey. Other than that, the presence of 2,3-butanediol, furfuryl alcohol, benzaldehyde and other sweet odorants such as γ-valerolactone, furfural and acetone in honey also contributed to the warm attribute of honey. In ginger, there were also two compounds reported to have warm attributes which were 4-hydroxy-2,5-dimethyl-3(2H)-furanone and vanillin. By comparing to the odorants that give rise to fresh and warm aroma in ginger, warm attributes decreased in ginger honey could be

explained by the types of odorants in ginger that lead to freshness are more than the odorants that give rise to warm aroma.

Based on Jiang et al. (2014), the major compound for astringency of a food is mainly from tannin. Despite the presence of 2-acetylfuran, furfural, benzaldehyde and octanal compounds, these compounds contributed to little astringent in original honey (Tian et al., 2018) whereas the ginger consists of more astringents including tannin, capsaicin, (E)-2-octenal, octanal, neral, geranial as reported by Babajide et al. (2013) and Schaller and Schieberle (2020a). This explained the higher astringency taste in ginger honeys of this study and was demonstrated in Figure 4.3.

As for the sweetness, the original honey scored the highest while the addition of ginger reduced the sweetness of honey as demonstrated in Figure 4.3. Based on Tian et al. (2018), the composition of honey including 3-methylbutanol, 1-pentanol, 2-ethylhexanol, γ-valerolactone, furfural, acetone, 2,3-pentenedione, geranic acid and 2-phenylacetaldehyde contributed to the sweetness of honey. Additionally, the reducing sugar content of honey, mainly fructose, is responsible for the sweetness of honey (Aljohar et al., 2018). Nonetheless, in this study, the reducing sugar content of original honey was the lowest but had the greatest sweetness intensity. This could be due to the original honey not being added with ginger, therefore sweetness dominated the flavour of original honey. However, though ginger honey had higher reducing sugar content as shown in Table 4.1, there were no odorants reported to contribute to the sweetness of ginger, but a great amount of odorants from ginger contributing to

the astringency, woody and spiciness flavour of ginger honey, hence the sweet aroma that could be sensed by panellist might be masked by these odorants in ginger honey, therefore reduced the sweetness scores in ginger honey (Schaller and Schieberle, 2020a).

On the basis of the minute bitterness in original honey contributed by 2-octanone and 2-acetylfuran compounds (Tian et al., 2018), the addition of ginger into honey resulted in increased bitter odorants including β-myrcene and 3-methyl-2-butene-1-thiol (Schaller and Schieberle, 2020a) from ginger into honey. This showed that the addition of ginger into honeys increased the bitterness of honey in this study which displayed in Figure 4.3. Moreover, the relationship between tannin concentration and pH value with bitterness of honey was demonstrated by a study by Otmani et al. (2019) in which the higher the concentration of tannin and pH value, the greater the intensity of bitterness of honey. The bitter taste and pH value among the honey samples in this study was corresponding to this relationship as shown in Table 4.1 and Figure 4.3 where the ginger honeys were evaluated with higher pH value and higher bitterness intensity. The taste attribute results obtained in this study were comparable to the study by Wilczyńska et al. (2017) where the higher the concentration of the spices added to the base honey, the higher the taste scores which specifically resulted in increased woody aroma including astringency, spiciness and resinous, bitterness, and pungent tastes but decreased the sweetness of the honey.

With regards to the persistence and aftertaste, all the honey samples showed higher persistence and aftertaste than sugar solution in Figure 4.3. This could be

due to higher viscosity and adhesiveness of honey which resulted from higher total sugar content as represented in Figure 4.3 and Table 4.2, eventually allowed honey to be trapped in the taste bud for a longer period until the honey was fully dissolved by saliva (Muñoz-González et al., 2022). Other than that, the addition of ginger into honey gave rise to strong flavours of ginger honey which linger on a person mouth and required longer time to be eliminated (Leonard, 2018), this explained the results obtained in this study where the higher the ginger concentration in ginger honey, the greater the persistence and aftertaste scores as shown in Figure 4.3.

5.1.3 Acceptability

Referring to Figure 4.4, it showed that the addition of ginger into honey had a negative impact on the acceptability of the honey. The original honey received the greatest acceptability chiefly for the reason that it had the highest sweetness and floral fresh fruit flavours, the least woody, astringency, sourness and bitterness flavours, absence of chemical attributes along with lighter colour intensity as displayed in Figure 4.1, Figure 4.2 and Figure 4.3. In contrast, ginger honeys were found to had lower acceptability and this could be due to the darker colour, higher concentration of ginger resulted in greater woody (spiciness, resinous), astringency, spoiled, sourness and bitterness flavours, and these attributes persisted in the mouth for longer time as compared to original honey. Similarly, there was a study proposed that the acceptability of spiced foods cannot be improved as the ‘sweetness threshold’ may not be achieved by solely adding spices to increase more flavour of the foods (Peters et al., 2018). The findings in this study was contradictory to the study by Wilczyńska et al. (2017)

which reported that increased concentration of the spices showed a higher degree of acceptance on the taste and aroma attributes of honey. Based on different acceptability scores among the honey samples, it can be suggested that the acceptability of honey was influenced by the visual, olfactory and taste attributes of the honey.

5.2 Physicochemical Properties

5.2.1 Colour Intensity

According to Saxena et al. (2010), the colour intensity of honey was proven to be related with its total phenolics content and antioxidant activity. The darker the honey, the greater the total phenolics content of honey, which resulted in greater antioxidant activity. In Table 4.1, the colour intensity of the three honey samples ranged from 207.67 mAU to 289.00 mAU, in which the ginger honey had a higher colour intensity from the original honey, which was 74.33 mAU higher, on average. The addition of ginger into honey had greater colour intensity might be due to higher total phenolics content presence in ginger honey thus higher antioxidant activity as shown in Table 4.4. This is comparable to the study reported by Čanadanović-Brunet et al. (2014), where the increased colour intensity was related to increased total phenolic content in honey. However, the colour intensity and total phenolics content did not show a similar trend for honey with 10% ginger and honey with 18% ginger whereby the later showed lighter colour intensity. This could be explained by the exposure to either high temperatures or light during the modification processes and different ways of handling the combs such as the use of old honeycombs and contact with metals that leads to changes in colour (Moniruzzaman et al., 2013b). Besides, the colour

intensity of honey also relies on the floral origin, mineral content, ash content and storage conditions of the honey (Ahmed et al., 2016a).

5.2.2 pH

Based on Table 4.1, the pH of the three honey samples varied at between 4.58 and 4.85 which showed the natural acidity of all the honey samples and were within the limit of pH 3.4 to 6.1 that indicates the freshness of honey (Moniruzzaman et al., 2013a). In this study, the pH of ginger honey in this study increased as the concentration of ginger increased and this could be explained by the acidity of ginger which is alkaline in nature and the transfer of the bitter odorants from ginger to honey (Purdie, 2019; Schaller and Schieberle, 2020a). Previous studies conducted by Bogdanov et al. (2007) and Moniruzzaman et al. (2013a) proposed that the high acidity of honey is due to the formation of organic acid by sugar fermentation in which is responsible for its flavour and its ability to cease growth of microorganism.

5.2.3 Total Sugar Content

According to Table 4.2, the total sugar content of the tested honey samples ranged from 78.70% to 81.30%, this showed higher values than previous studies by Moniruzzaman et al. (2013a) in which the total sugar content of honeybee honey ranged from 63.33% to 68.40%. Nonetheless, the addition of 18% ginger to honey caused the reduction of total sugar content as shown in Table 4.2, where the total sugar content of honey with 18% ginger was the lowest as compared to original honey which showed a 1.4% difference. This result shared similarity to

a study conducted by Babajide et al. (2013) where the reduction was shown in the total sugar content from 8.08% to 7.60% as the spice concentration increased (Babajide et al., 2013). These findings were supported by studies which reported that the spices including ginger, cinnamon, cardamom, clove, nutmeg, anise and mace have the ability to eliminate or decrease the sugar content in foods (Babajide et al., 2013; Peters et al., 2018).

5.2.4 Reducing Sugar Content

As shown in Table 4.2, the reducing sugar content of the honey samples ranged from 59.53% to 63.27%. In comparison to previous studies by Moniruzzaman et al. (2013a) and Moniruzzaman et al. (2013b), which reported that the range of reducing sugar content in honey bee honey were 61.17% to 63.89% and 52.17% to 62.17%, respectively, the range of reducing sugar content results from this study were wider. This demonstrated that the addition of ginger increased the reducing sugar content of the honey, this could be due to the transfer of reducing sugar from ginger to honey (Lee et al., 2014). Based on Lee et al. (2014), ginger consists of 2.2% (w/w) of reducing sugars which contributed to minor amounts of increment in reducing sugar content in ginger honey. This was shown in this study where the ginger concentration in honey increased, the reducing sugar content of honey increased.

5.2.5 Moisture Content

As shown in Table 4.2, the moisture content of the honey samples ranged between 18.70% to 21.30%. In comparison to the study conducted by Moniruzzaman, et al. (2013a) which reported that the moisture content of

honeybee honey ranged between 14.86% to 17.53%, the data obtained from this study was higher. The relationship between total sugar content and moisture content was shown to be inversely proportional in this study as shown in Table 4.2 and was supported by another research which proposed that the high total sugar content of the honey was attributed to the low moisture content (Moniruzzaman et al., 2013a). Additionally, according to Yanniotis et al. (2006), the moisture content of honey determines the viscosity of honey. Referring to Figure 4.1, the honey with 10% ginger displayed the highest viscosity which could be explained by the lowest moisture content in honey with 10% ginger as shown in Table 4.2.

5.2.6 Water Activity

In Table 4.2, original honey and honey with 10% ginger shared the same value for water activity which was 0.54 while honey with 18% ginger showed higher water activity which was 0.55. In comparison to other studies, the values were lower than the reported values for other honeybee honeys which ranged between 0.56 and 0.62 (Olaitan, et al., 2007). Besides, the reason for honey with 18% ginger having the highest water activity could be due to the addition of 18% ginger to the honey, resulting in decreased total sugar content which leads to increment of the moisture content of honey, as reported in Table 4.2. This was supported by the reports proposed by Abramovič et al. (2008) and Chen (2019) which discussed the water activity has a mutual relationship with moisture content of honey. This was shown in Table 4.2, in which honey with 18% ginger exhibited the highest water activity and moisture content as compared to other honey samples. Furthermore, the water activity in all the tested honey samples

was lower than 0.60, this indicated that the honey samples were less likely to be susceptible to microbial fermentation. This is because the reported water activity requirement for the mould, yeast and bacteria to grow were 0.70, 0.80 and 0.90, correspondingly (Jimenez et al., 2016; De-Melo et al., 2018).

5.2.7 Electrical Conductivity

Based on Table 4.3, the electrical conductivity of tested honey samples was in the range of 290.33 μS/cm to 535.67 μS/cm. In comparison with original honey, the addition of ginger affected the electrical conductivity of honey which caused an increase of 1.54 times in honey with 10% ginger and 1.85 times in honey with 18% of ginger. This could be due to the combination of ash content from honey and ginger. The reported ash content in honey ranged from 0.23% to 2.33% (El Sohaimy and Shehata, 2015) whereas the reported ash content in ginger was 7.64%, the combination of these two generated higher ash content in ginger honey which resulted in greater electrical conductivity in ginger honey (Latona et al., 2012). To support this, previous studies showed the relationship between ash content and electrical conductivity, which is the greater the ash content, the greater the electrical conductivity of honey (Yadata, 2014; Sereia et al., 2017). In addition, previous studies also proved that the floral origin, mineral content, amount of organic acids and proteins were related to the electrical conductivity of honey (Acquarone et al., 2007; Karabagias et al., 2014).

5.3 Antioxidant Properties

Since phenolic substances have been shown to be responsible for the antioxidant activity of honey, the total phenol content of the honey samples were

investigated. Referring to results in Table 4.4, the total phenolic content of honey with 10% ginger and honey with 18% ginger increased 2.10 times and 2.58 times, respectively, compared to original honey. This could be explained by the transfer of the bioactive compounds including gingerol, shogaol and paradol (Mao et al., 2019) from ginger to honey that caused an increase in total phenolic content of the ginger honeys as reported previously by another study, where the addition of prunes into honey increased the antioxidant activity when the bioactive compounds of prunes were transferred to honey (Čanadanović-Brunet et al., 2014). Furthermore, according to Dżugan et al. (2018), the total phenolic content is related to the honey colour. The greater the colour intensity of honey, the higher the total phenolics content, which indicated greater antioxidant activity. This relationship was shown in this study, where the ginger honey possessed darker colour (Table 4.1) and greater total phenolic content (Table 4.4) compared to the original honey. Additionally, Wilczyńska et al. (2017) reported that the elevated free-radical (DPPH) scavenging capacity, from 56% to a range of 58% to 90%, observed in multifloral honey and honey added with different types and concentrations of spices was due to the increased level of phenolic compounds from 43 mg GAE/100g to a range of 45 mg GAE/100g to 90 mg GAE/100g. Similarly, this finding was displayed in this study where the superoxide anion radical scavenging activity and hydroxyl radical scavenging activity among the tested honey samples were increased as the total phenolic content increased in ginger honeys as demonstrated in Table 4.4.

The greatest increase of antioxidant activity was noted for iron chelating activity, where addition of 10% and 18% of ginger in honey increased 4.52 times and

4.67 times of iron chelating activity in honey, respectively, compared to original honey. As seen from Table 4.4, original honey revealed the weakest iron chelating activity for antioxidant properties of honey in this study which was compatible to studies by Bellik and Selles (2016), which reported that the iron chelating activity of multifloral Algeria honey was low in which the multifloral Algeria honey exhibited less than 10% of iron chelating activity, this resulted in low contribution to antioxidant activity of honey samples. In contrast, the ginger honeys in this study exhibited stronger iron chelating activity which could be attributed to the presence of more flavonoids in the ginger honeys. Based on Hatcher et al. (2009) and Wang et al. (2021), the flavonoids contents are beneficial as iron chelators. In addition to the flavonoids present in honey such as quercetin, kaempferol, apigenin, pinocembrin and galangin, there are flavonoids also present in ginger including quercetin, rutin, baicalein, kaempferol, naringenin and catechin in which the former three are reported to have the strongest ability to chelate iron (Sun et al., 2008; Wang et al., 2021). Therefore, the addition of ginger into honey had the greatest positive impact on iron chelation due to the combination of flavonoids from ginger and honey in this study.

In parallel, the influence of ginger on superoxide anion radical scavenging activity of honey was observed in this research as summarised in Table 4.4, there was a 1.48 fold and 1.52 fold increase in superoxide anion radical scavenging activity by honey with 10% ginger and honey with 18% ginger, respectively, in comparison with original honey. This showed the superoxide free radicals were scavenged in a concentration-dependent manner where the higher the ginger

concentration, the greater the superoxide anion radical scavenging activity. Other than the reason the ginger honey consists of higher total phenolic content as shown in Table 4.4, the presence of flavonoids would influence the superoxide anion radical scavenging activity by ginger and honey as well (Lalhminghlui and Jagetia, 2018; Kishore et al., 2011). This was supported by studies, where the authors concluded that flavonoids increase the scavenging of superoxide anion radical and the reported flavonoids were kaempferol, morin, catechin, quercetin, myricetin and rutin that present in ginger and/or honey (Robak and Gryglewski, 1988; Gandhimathi et al., 2011)

In addition, as displayed in Table 4.4, the hydroxyl radical scavenging activity of honeys ranged between 70.24% to 72.88% which showed slight differences, where honey with 18% ginger increased 1.03 fold compared to original honey. The increase in hydroxyl radical scavenging activity was correlated with the total phenolic content in honeys as shown in Table 4.4, the higher the total phenolic content, the higher the hydroxyl radical scavenging activity. This relationship was in accordance with study by Zhou et al. (2012), in which the researchers proposed that there were strong correlations between hydroxyl radical scavenging activity with total phenolic content. Meanwhile, Lalhminghlui and Jagetia (2018) reported that the scavenging of hydroxyl radical was facilitated by flavonoids in a concentration-dependent manner. Several flavonoids including catechin, myricetin, quercetin, kaempferol that present in honey and/or ginger have been reported to scavenge hydroxyl free radicals (Treml and Šmejkal, 2016).

In overall, the findings in this study proved that addition of ginger had positive effects on antioxidant activities of ginger honey, which was comparable to the study of Wilczyńska et al. (2017) showed that addition of 0.5%, 1% to 2% concentration of ginger resulted in increased total phenolics content and DPPH radical scavenging activity. These findings were supported by another study which showed the total phenolics content increased with increased concentration (20%, 30% and 40%) of prunes in honeys (Čanadanović-Brunet et al., 2014). Other than that, the DPPH, hydroxyl and superoxide anion radical scavenging activity demonstrated a similar trend with increased total phenolics content that the honey with higher concentrations of prunes exhibited greater antioxidant activity as compared to honeys without prunes (Čanadanović-Brunet et al., 2014).

5.4 Antibacterial Properties

Original honey and ginger honey exerted inhibitory activity on gram-positive and gram-negative bacteria tested on this study. This could be explained by the presence of antibacterial properties of ginger and honey including acidity, osmolarity, hydrogen peroxide, water activity, total phenolic and flavonoid contents (Ng et al., 2014; Mao et al., 2019). According to Bizerra et al. (2012), the synergistic antibacterial effect of honey can be attributed to the interaction between hydrogen peroxide and other compounds of honey including antioxidants and traces of transition metals in honey, which modulating the oxidative effect of honey. In the presence of oxygen, the polyphenols will be oxidised and subsequently generate hydrogen peroxide. When hydrogen peroxide react with transition metal in honey, hydroxyl radicals will be produced

via Fenton reaction. In consequence, this interaction results in the oxidation of biomolecules which give rise to DNA degradation and bacterial growth inhibition (Ng et al., 2021). Other than that, the finding showed that the original honey was not effective against *Enterococcus faecalis* could be due to the thick peptidoglycan contained in gram-positive bacteria to resist the damaging action of original honey (Silhavy et al., 2010). Meanwhile, the addition of ginger into honey enhanced the inhibition of *E. faecalis* could be explained by the synergistic antimicrobial effect of ginger honey. Omaya and Akharaiyi (2011) reported that the ginger were positive for identified antimicrobial agents such as flavonoids, phlobatannin, saponin, alkaloids and cardiac glycosides. Meanwhile, Gheldof et al. (2002) proposed that the antimicrobial properties of honey could be due to the phytochemicals including phenolic content and flavonoids. Hence, the synergistic antimicrobial impact of ginger honey might be associated with the increase in the amount of flavonoids in the mixture since honey and ginger contained these antimicrobial agents (Ewnetu et al., 2014). This was comparable to a study by Ewnetu et al. (2014), where the synergistic effect of honey ginger mixture on antimicrobial effects on *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* were shown in which the use of honey and ginger extracts individually were found to have average zone of inhibition of 21.63 mm ± 3.30 and 19.23 mm ± 3.42, respectively, whereas the mixture of honey and ginger extracts showed a higher average zone of inhibition which was 25.62 mm ± 2.55. Furthermore, *Pseudomonas aeruginosa* was highly susceptible to both honey and ginger honey with no significant difference. Possible reason could be due to the lesser peptidoglycan of gram-negative bacteria hence causing the

gram-negative bacteria to be more sensitive to damaging action (Tortora et al., 2013).

5.5 Future Studies

Further recommendations for this study includes determination of flavonoids content in ginger honey with high performance liquid chromatography (Ghasemzadeh et al., 2010) to investigate the type and amount of flavonoids present in ginger honey and to evaluate the relationship between flavonoids content and antioxidant activity of ginger honey. Subsequently, the measurement of hydroxymethylfurfural content (HMF) and proline could be incorporated into future studies, where the HMF serve as freshness indicator (Shapla et al., 2018) while proline serves as a maturity or adulteration indicator of honey (De-Melo et al., 2018). In addition, evaluation of ash content could be performed as this parameter can be used to correlated with electrical conductivity (Yadata, 2014) and colour intensity (Bertoncelj et al., 2007). Last but not least, the measurement of hydrogen peroxide level in honey could be included in future study to examine the effect of ginger on hydrogen peroxide level, where the antibacterial property of honey varies among different concentration of hydrogen peroxide.

CHAPTER 6

CONCLUSION

In conclusion, the sensory analysis findings had suggested that the addition of ginger influenced the visual, texture, olfactory, taste and acceptability attributes of the honey, in which the addition of ginger increased woody (spiciness, resinous), fresh, astringency and bitterness attributes but decreased sweetness, floral fresh fruit, warm, persistence and aftertaste attributes of the honey. Due to this, the acceptability of ginger honey was reduced. Furthermore, the addition of ginger resulted in increased colour intensity, moisture content, water activity, electrical conductivity, pH and reducing sugar content whereas the total sugar content was reduced. The significant changes on physicochemical parameters were observed when the honey added with different concentration of ginger which representing the greater discrimination capability between the honey samples. Besides, the addition of ginger into honey had a significant contribution on the increment of total phenolic content, iron chelating activity, superoxide anion scavenging activity and hydroxyl radical scavenging activity. Moreover, the synergistic antibacterial effect of the ginger honey was shown, where the original honey was unable to exhibit inhibitory effect against *Enterococcus faecalis* whereas the addition of ginger into honey, was more effective in inhibiting the growth of *E. faecalis*. In contrast, the addition of ginger had no significant impact on antibacterial effect of honey against *Pseudomonas aeruginosa*.

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Appendices

Appendix A

Figure A: Honey samples from left to right are original honey, honey with 10% ginger and honey with 18% ginger

Appendix B

Table A: List of chemicals and media used with 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 Absolute (99.9 %)	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
L-ascorbic acid	GENE Chem, France
MacConkey agar	Chem Soln, India
Mannitol salt agar	Condalab, Spain
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

Appendix C

Table A: List of 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
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, UK

Appendix D

Reagent and media preparations

Ampicillin solution (10 µg/mL)

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

Ascorbic acid solution (1.8mM)

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

Deoxyribose solution (50mM)

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

Dipotassium phosphate (K_2HPO_4) (1M)

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

Ferric chloride solution (3.2mM)

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

Ferrous sulphate solution (1mM)

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

Ferrozine solution (0.25mM)

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

NADH (2.34mM)

NADH solution was prepared by dissolving approximately 0.0166 g of NADH powder into the Tris buffer with a final volume of 10 mL.

Na_2EDTA solution (1mM)

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

Nitroblue tetrazolium (NBT) stock solution

NBT solution was prepared by dissolving 10 mg of NBT tablet in 1 mL of distilled water.

Nitroblue tetrazolium (NBT) (0.78mM)

NBT solution was prepared by dissolving approximately 64 µL of NBT stock solution into 936 µL of distilled water then topped up to a final volume of 1000 µL.

Phenazine methosulfate (0.06mM)

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

Phosphate buffer (100mM, pH 7.4)

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

Potassium dihydrogen phosphate (KH_2PO_4) (1M)

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

Rochelle salt [40% (w/v)]

Rochelle salt was prepared by dissolving 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 dissolving approximately 5 g of sodium carbonate into 50 mL of distilled water.

Sodium hydroxide (NaOH) solution (1.25%)

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

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

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

Tris buffer (0.1 M, pH 8)

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

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

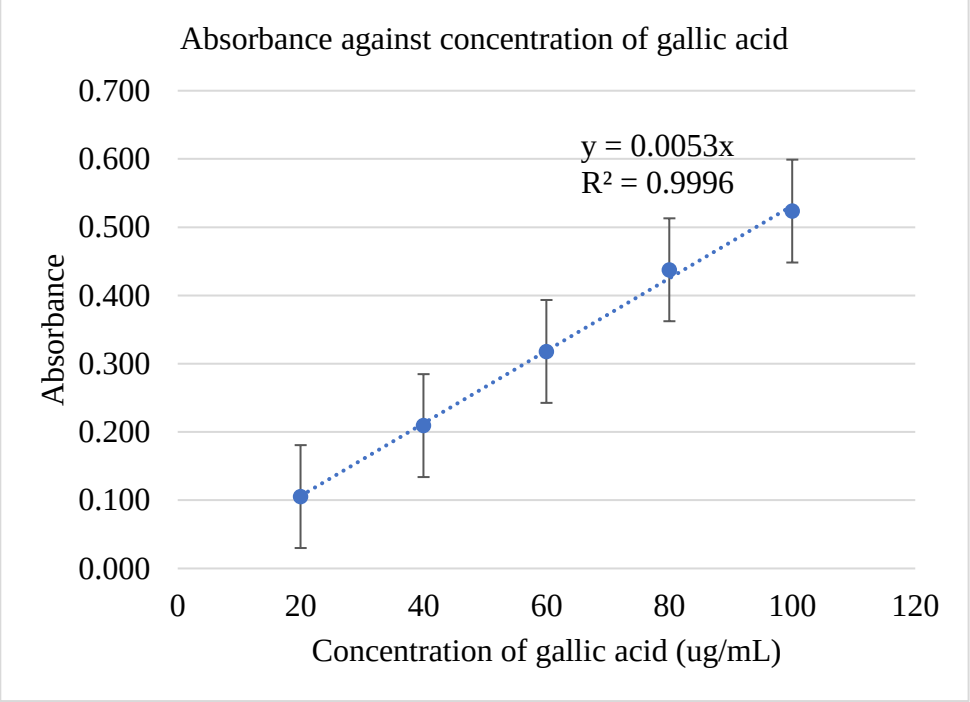

Figure A: Graph of absorbance against concentration of gallic acid.

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

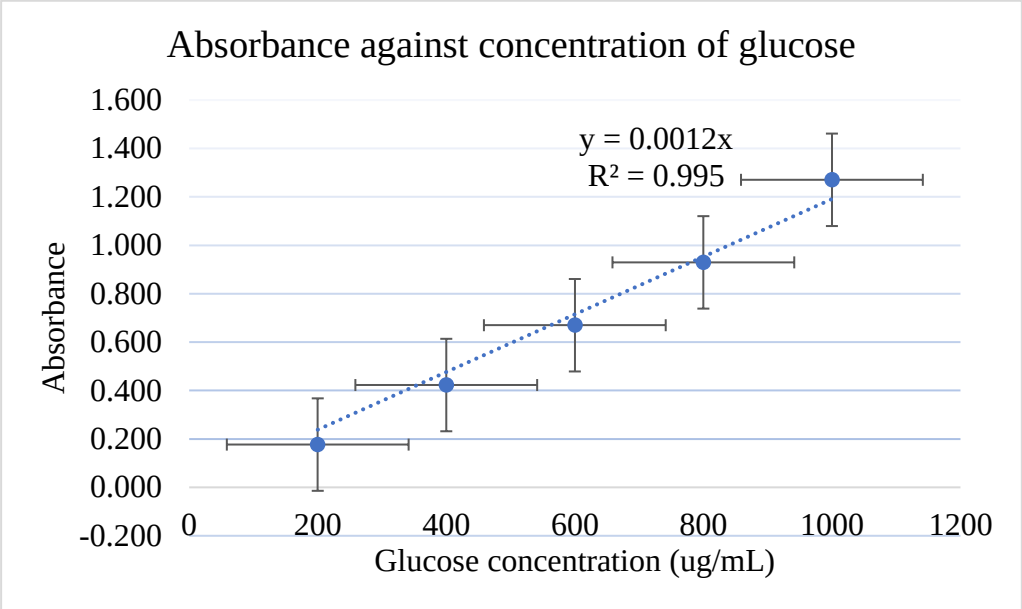

Figure B: Graph of absorbance against concentration of glucose.

Appendix F

Sensory evaluation guidelines for the panellists

Attributes to be evaluated:

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)

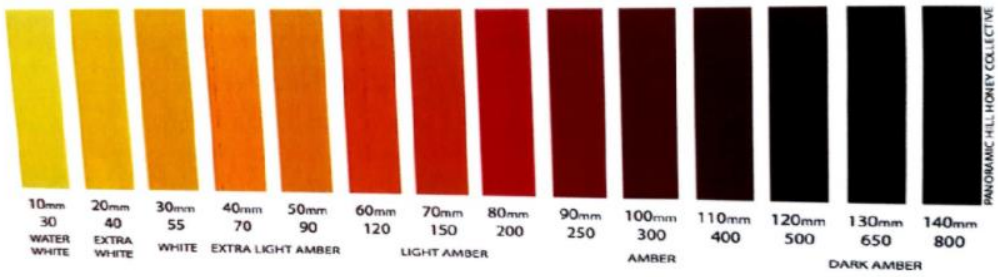

Figure A: USDA Honey colour grading chart for colour intensity attribute

(For colour intensity rating: 1-white; 2-extra light amber; 3-light amber; 4-amber; 5-dark amber)

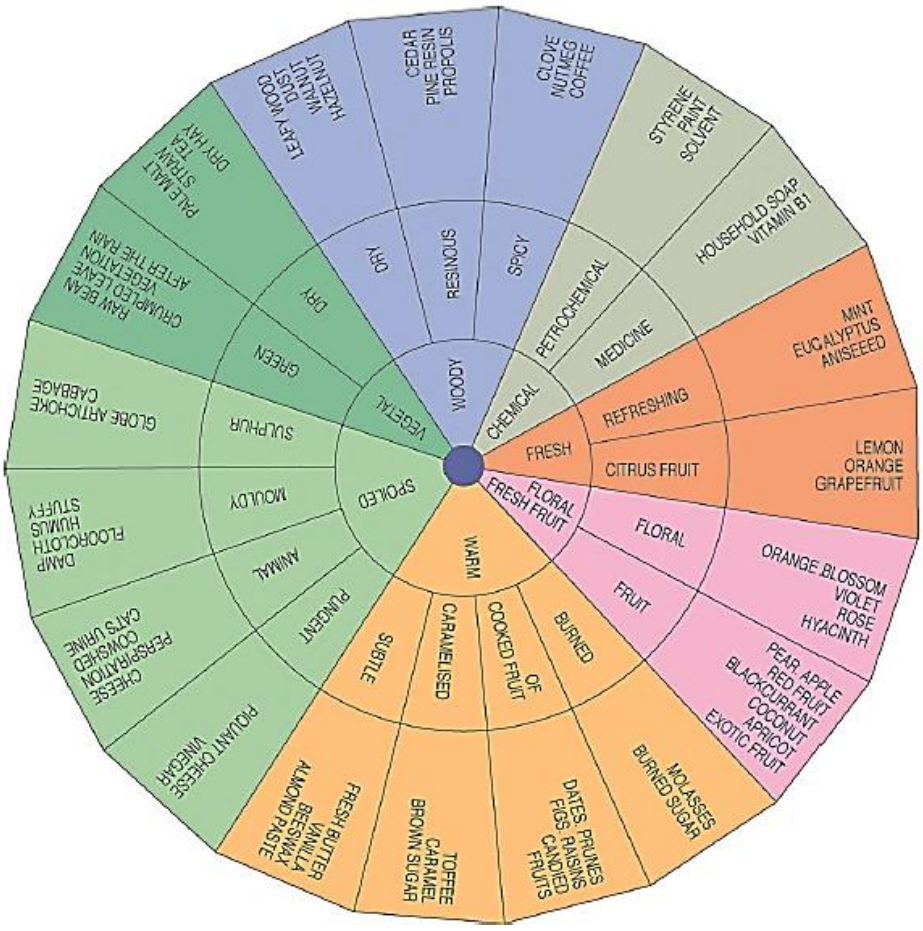

Figure B: Odour and aroma wheel for olfactory and taste attributes

Explanation of 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.

Appendix G

Master sheet and score sheet for sensory analysis

Master sheet

Master Sheet

Panel Number: _____ Date: _____

Samples	**A**	**B**	**C**	**D**
Code				
Permutation				

Score sheet

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				

Appendix H

Sensory analysis results

Table A: The percentage score for sensory attributes of sugar solution, original honey, honey with 10% ginger and honey with 18% ginger.

Attributes	Percentage score (%)			
	SS	**H1**	**H2**	**H3**
Visual				
Colour intensity	57.5	50	62.5	67.5
Viscosity	22.5	70	92.5	67.5
Olfactory				
Woody	0	25	62.5	62.5
Chemical	12.5	0	0	12.5
Fresh	0	37.5	75	75
Floral fresh fruit	0	75	75	50
Warm	12.5	62.5	50	37.5
Spoiled	0	0	0	0
Vegetal	25	50	37.5	12.5
Taste				
Woody	0	25	62.5	62.5
Chemical	37.5	0	0	0
Fresh	12.5	37.5	75	50
Floral fresh fruit	50	75	62.5	37.5
Warm	50	62.5	50	50
Spoiled	0	0	0	12.5
Vegetal	12.5	25	50	50
Astringency	12.5	25	75	75
Refreshing	12.5	50	100	87.5
Sweetness	55	80	70	55
Sourness	20	30	35	35
Saltiness	20	30	30	32.5
Bitterness	20	25	42.5	40
Persistence	27.5	67.5	75	82.5
After taste	22.5	52.5	77.5	87.5
Texture				
Adhesiveness	20	72.5	87.5	75
Acceptability	27.5	90	67.5	55

Appendix I

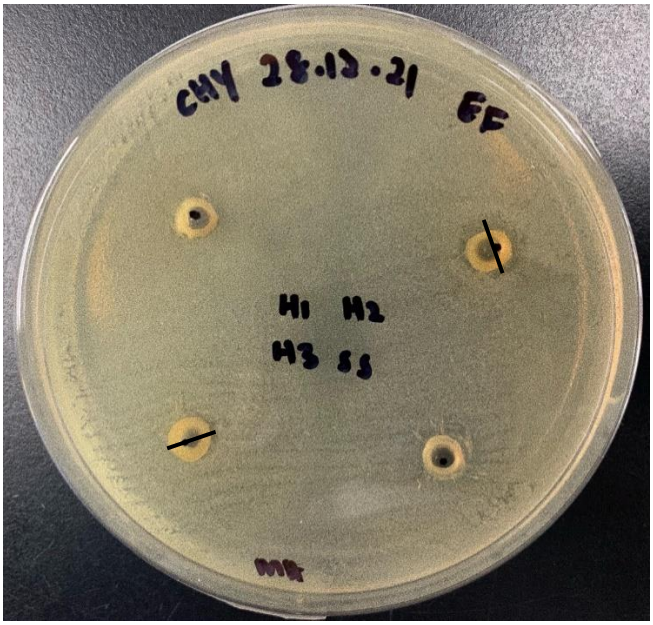

Figure A: Zone of inhibition (indicated by black line) of *Enterococcus faecalis* ATCC 29212 for original honey (H1), honey with 10% ginger (H2) and honey with 18% ginger (H3) and sugar solution (SS).

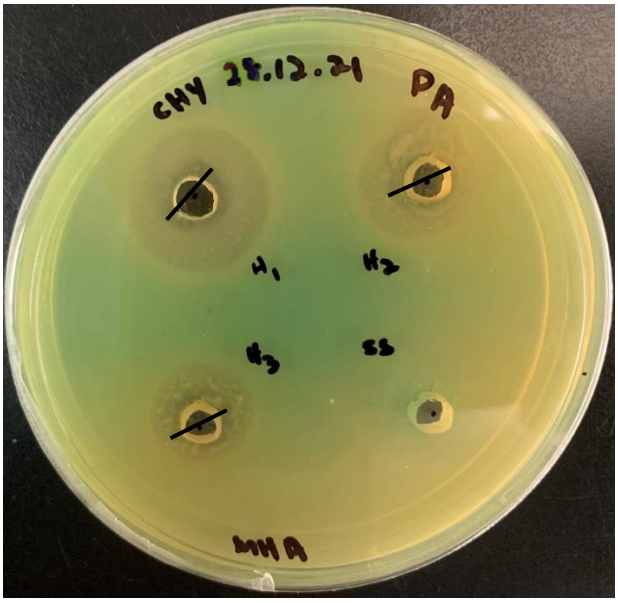

Figure B: Zone of inhibition (indicated by black line) of *Pseudomonas aeruginosa* ATCC 27853 for original honey (H1), honey with 10% ginger (H2) and honey with 18% ginger (H3) and sugar solution (SS).

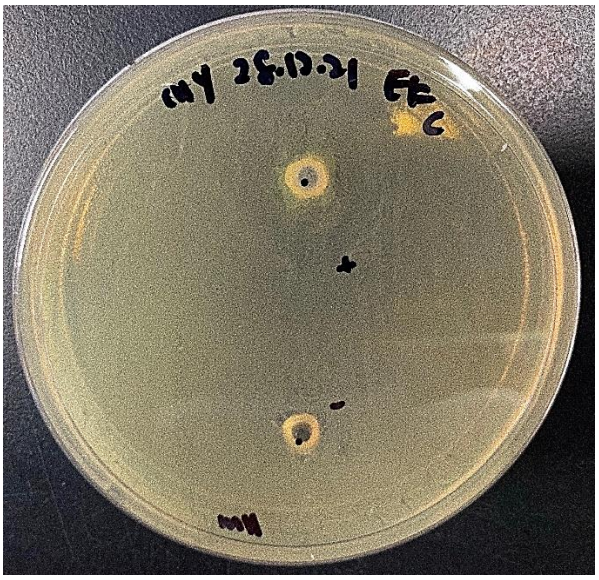

Figure B: Example of control plate of *Enterococcus faecalis* ATCC 29212 for 10 µg/mL ampicillin solution as positive control (top) and distilled water as negative control (bottom).

Appendix J

ANOVA test for physicochemical, antioxidant and antibacterial assays

Table A: Statistical analysis for physicochemical properties of original honey (H1), honey with 10% ginger (H2) and honey with 18% ginger (H3).

Dependent Variable	(I) Honey Sample	(J) Honey Sample	Mean Difference (I-J)	Sig.
Colour Intensity (mAU)	H1	H2	-81.500*	<.001
		H3	-67.500*	<.001
	H2	H1	81.500*	<.001
		H3	14.000*	.033
	H3	H1	67.500*	<.001
		H2	-14.000*	.033
Water Activity	H1	H2	.000333	.968
		H3	-.013667*	<.001
	H2	H1	-.000333	.968
		H3	-.014000*	<.001
	H3	H1	.013667*	<.001
		H2	.014000*	<.001
Electrical Conductivity (µs/Cm)	H1	H2	-155.666667*	<.001
		H3	-245.333333*	<.001
	H2	H1	155.666667*	<.001
		H3	-89.666667*	<.001
	H3	H1	245.333333*	<.001
		H2	89.666667*	<.001
pH	H1	H2	-.260000*	<.001
		H3	-.276667*	<.001
	H2	H1	.260000*	<.001
		H3	-.016667	.258
	H3	H1	.276667*	<.001
		H2	.016667	.258
Reducing Sugar Content (%)	H1	H2	-1.310000*	.025
		H3	-3.953333*	<.001
	H2	H1	1.310000*	.025
		H3	-2.643333*	<.001
	H3	H1	3.953333*	<.001
		H2	2.643333*	<.001

*. The mean difference is significant at the 0.05 level.

Table B: Statistical analysis for antioxidant properties of original honey (H1), honey with 10% ginger (H2) and honey with 18% ginger (H3).

Dependent Variable	(I) Sample	(J) Sample	Mean Difference (I-J)	Sig.
Total Phenolics Content (mg GAE/kg)	H1	H2	-18.144667*	<.001
		H3	-26.038000*	<.001
	H2	H1	18.144667*	<.001
		H3	-7.893333*	<.001
	H3	H1	26.038000*	<.001
		H2	7.893333*	<.001
Iron Chelating Activoty (%)	H1	H2	-19.15333*	<.001
		H3	-20.01667*	<.001
	H2	H1	19.15333*	<.001
		H3	-.86333	.701
	H3	H1	20.01667*	<.001
		H2	.86333	.701
Superoxide Anion Scavenging Activity (%)	H1	H2	-27.30000*	<.001
		H3	-29.87000*	<.001
	H2	H1	27.30000*	<.001
		H3	-2.57000*	.019
	H3	H1	29.87000*	<.001
		H2	2.57000*	.019
Hydroxyl Radical Scavenging Activity (%)	H1	H2	-.29333	.416
		H3	-2.64000*	<.001
	H2	H1	.29333	.416
		H3	-2.34667*	<.001
	H3	H1	2.64000*	<.001
		H2	2.34667*	<.001

*. The mean difference is significant at the 0.05 level.

Table C: Statistical analysis for antibacterial properties of original honey (H1), honey with 10% ginger (H2) and honey with 18% ginger (H3).

Dependent Variable	(I) Sample	(J) Sample	Mean Difference (I-J)	Sig.
Zone of Inhibition_PA	H1	H2	-.01000	.997
		H3	-.04500	.805
	H2	H1	.01000	.997
		H3	-.03500	.891
	H3	H1	.04500	.805
		H2	.03500	.891
Zone of Inhibition_EF	H1	H2	-.83333*	<.001
		H3	-.75000*	<.001
	H2	H1	.83333*	<.001
		H3	.08333	.391
	H3	H1	.75000*	<.001
		H2	-.08333	.391

*. The mean difference is significant at the 0.05 level.

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