IDENTIFICATION OF LACTIC ACID BACTERIA, PHYSICOCHEMICAL PROPERTIES AND ANTIBACTERIAL ACTIVITIES OF MALAYSIAN *Heterotrigona itama* HONEY

SYARIFFAH NURATIQAH SYED YAACOB

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Philosophy

Faculty of Science
Universiti Teknologi Malaysia

DECEMBER 2018

DEDICATION

Specially dedicated to:

My beloved Papa and Umi for their endless motivation and prayers

ACKNOWLEDGEMENT

First and foremost, I am expressing the uttermost gratitude to the Al-Mighty Allah for showering His endless blessings, guidance, wisdom, good health upon me to successfully complete the research. May His Name be praised for ever.

Special appreciation to my beloved co-supervisor, Assoc. Prof. Dr. Roswanira Abdul Wahab for her unwavering support and mentorship throughout this research. Her commitment and sincerity with her duties really inspired me a lot. Without her constant motivation, guidance and productive criticism, it might not have been possible for me to complete the research.

I would like to express my warmest gratitude to Dr. Raja Kamarulzaman Raja Ibrahim, Prof. Dr. Fahrul Huyop and Dr. Nizam Lani for being supportive throughout this research. Their enthusiast, passion, responsibility, fairness, professional ethics in doing research impress me deeply.

My cordial gratitude to my family for their inexhaustible source of inspiration and not to forget the members of Microbiology Research Laboratory and Biotechnology and Biochemistry Laboratory for their warm help throughout my research.

ABSTRACT

Honey produced by Malaysian stingless bee, *Heterotrigona itama* (*H. itama*) is highly valued for its numerous therapeutic benefits. Despite the extensive studies on lactic acid bacteria (LAB), data on LAB from raw Malaysian *H. itama* honey is unavailable. Moreover, there is a huge demand for novel antibacterial agents as the issue of multi-drug resistant bacteria is on the rise. Natural compounds such as honey may serve as a possible source of reliable solutions to this problem. Herein, this study reports the isolation and identification of LAB as well as physicochemical properties, antioxidant and antibacterial activities of *H. itama* honey against pathogenic bacteria. This study also reports the changes in the nutritional properties of *H. itama* honey by assessing the changes in LAB population, physicochemical aspects and antioxidant activity. Four LAB strains, Sy-1, Sy-2, Sy-3 and Sy-4 were successfully isolated from raw *H. itama* honey. Based on 16S rRNA analysis, strain Sy-1 is closely related with the Lactobacillus genus (>91%) while strains Sy-2, Sy-3 and Sy-4 are phylogenetically grouped in the *Fructobacillus fructosus* subcluster with sequence similarities of 98%, 96% and 95%, respectively. API 50 CHL test showed that these LAB strains preferred fructose and glucose as substrates. The LABs showed good antibacterial activities against *Bacillus subtilis, Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Physicochemical analysis demonstrated that *H. itama* honey has lower pH (2.7 ± 0.19) but higher in moisture (30.3 ± 0.56%) and protein content (15156 ± 2 mg/kg), than the honey of sting bee *Apis mellifera*. HPLC data showed *H. itama* honey has low glucose (12.05 ± 0.54 g/100 g) and fructose contents (9.94 ± 0.43 g/100 g), high in maltose (25.7 ± 1.66 g/100g) and absent of sucrose. Antioxidant activities of the honey were significantly correlated to phenolic contents ($p < 0.01$) but were moderately associated to colour and flavonoid contents. Agar well diffusion assay showed *H. itama* honey are more potent against Gram-positive bacteria (31.38 ± 3.12 mm) than Gram-negative bacteria (23.75 ± 5.13 mm). Importantly, honey Sy-1 exhibited the strongest antioxidant and antibacterial activities amongst the honey samples. A 28-day storage duration test of the *H. itama* honey showed that viability of LAB population in honey reduced significantly ($p < 0.05$), meanwhile pH, moisture content, phenolic and flavonoid contents as well as antioxidant capacity remained relatively stable ($p > 0.05$). This study demonstrated that Malaysian *H. itama* honey is not only a valuable reservoir for new LAB, but it also preserves probiotic properties and is rich in nutritious elements. Thus, the findings support the potential therapeutic use of LAB isolated from raw Malaysian *H. itama* honey in treating various ailments.

ABSTRAK

Madu yang dihasilkan oleh lebah *Heterotrigona itama* (*H. itama*) Malaysia sangat dihargai untuk pelbagai manfaat terapeutiknya. Walaupun kajian mengenai bakteria asid laktik (LAB) adalah sangat meluas, namun tiada data yang dilaporkan mengenai LAB daripada madu *H. itama* yang terdapat di Malaysia. Selain itu, terdapat permintaan yang tinggi untuk agen antibakteria baru kerana isu bakteria perintang pelbagai ubat semakin meningkat. Sebatian semulajadi seperti madu boleh dijadikan sumber penyelesaian yang boleh dipercayai untuk masalah ini. Di sini, kajian ini melaporkan pemencilan dan pengenalpastian LAB serta sifat fizikokimia, aktiviti antioksidan dan antibakteria *H. itama* madu terhadap bakteria patogen. Kajian ini juga melaporkan perubahan dalam sifat nutrisi madu *H. itama* dengan menilai perubahan dalam populasi LAB, aspek fizikokimia dan aktiviti antioksidan. Empat strain LAB iaitu Sy-1, Sy-2, Sy-3 dan Sy-4 berjaya dipencilkan daripada madu *H. itama*. Berdasarkan analisis 16S rRNA, strain Sy-1 menunujukkan hubungan yang rapat dengan genus *Lactobacillus* (> 91%) sedangkan strain Sy-2, Sy-3 dan Sy-4 secara filogenetik telah disubkluster didalam kumpulan *Fructobacillus fructosus* dengan urutan kesamaan masing- masing 98% 96% dan 95%. Ujian API 50 CHL menunjukkan bahawa semua strain LAB lebih memilih fruktosa dan glukosa sebagai substrat. LAB menunjukkan aktiviti antibakteria terhadap *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa dan Escherichia coli*. Analisis fizikokimia menunjukkan bahawa madu *H. itama* mempunyai pH yang lebih rendah (2.7 ± 0.19), kelembapan (30.3 ± 0.56%) dan kandungan protein (15156 ± 2 mg / kg) yang lebih tinggi berbanding madu lebah *Apis mellifera*. Data HPLC menunjukkan bahawa madu *H. itama* mempunyai kandungan glukosa (12.05 ± 0.54 g / 100 g) dan fruktosa (9.94 ± 0.43 g / 100 g) yang rendah, maltosa (25.7 ± 1.66 g / 100g) yang tinggi dan tiada sukrosa. Aktiviti antioksidan madu berkadaran secara signifikan dengan kandungan fenolik ($p < 0.01$) namun berkadaran secara sederhana dengan warna dan kandungan flavonoid. Ujian peresapan agar-agar menunjukkan bahawa madu *H. itama* lebih berkesan terhadap bakteria Gram-positif (31.38 ± 3.12 mm) daripada bakteria Gram-negatif (23.75 ± 5.13 mm). Madu Sy-1 mempamerkan aktiviti antioksidan dan antibakterial paling tinggi di antara sampel-sampel madu. Ujian tempoh penyimpanan madu *H. itama* selama 28 hari menunjukkan pengurangan kandungan LAB yang signifikan ($p < 0.05$), sementara kandungan pH, kadar kelembapan, kandungan fenolik dan flavonoid serta kapasiti antioksidan kekal stabil ($p > 0.05$). Kajian ini menunjukkan bahawa madu *H. itama* Malaysia bukan sahaja merupakan takungan yang berharga untuk LAB, malah ia menunjukkan sifat-sifat probiotik dan kaya dengan unsur nutrisi. Oleh itu, penemuan ini menyokong keupayaan penggunaan terapeutik LAB yang terdapat dalam madu *H. itama* Malaysia dalam merawat pelbagai penyakit.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	-	Analysis of Variance
BLAST	-	Basic Local Alignment Search Tool
BSA	-	Bovine Serum Albumin
DPPH	-	2,2-Diphenyl-1-picrylhydrazyl
EDTA	-	Ethylenediaminetetraacetic Acid
EtBr	-	Ethidium Bromide
FRAP	-	Ferric Reducing Antioxidant Power
HMF	-	Hydromethylfurfural
MEGA6	-	Molecular Evolutionary Genetic Analysis Software
MIC	-	Minimum Inhibitory Concentration
MBC	-	Minimum Bactericidal Concentration
PCR	-	Polymerase Chain Reaction
SEM	-	Scanning Electron Microscopy
TFC	-	Total Flavonoid Content
TFC	-	Total Phenolic Content
UV	-	Ultraviolet
UV-Vis	-	Ultraviolet-Visible
16S rRNA	-	16 Subunit Ribosomal Deoxyribonucleic Acid

LIST OF SYMBOLS

%	-	Percentage
°C	-	Celsius
bp	-	Base pair
Ca	-	Calcium
h	-	Hour
kg	-	Kilogram
min	-	Minute
mL	-	Millilitre
mM	-	Millimolar
NaCl	-	Sodium Chloride
NaOH	-	Sodium Hydroxide
ng	-	Nanogram
rpm	-	Revolution per minute
s	-	Second
v/v	-	Volume percentage per 100 mL volume
w/v	-	Weight per volume percentage
µmol	-	Micromole
µg	-	Microgram

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Honey consists of complex constituent that is widely known as reservoir of essential nutrients. These nutrients originate from the gathering activity of plant nectar and then released following their digestion by the honey stomach of the bees. There are numerous claims on the outstanding therapeutic profile of honey with this commodity showing activities associated with antibacterial, anti-inflammatory, anti-diabetic, antioxidant, as well as a source of swift energy booster and obesity reducer (Al-Waili and Haq, 2004; Fukuda *et al.,* 2011; Yaghoobi *et al.,* 2008; Zainol *et al.,* 2013). Besides being the health-promising dietary complement, a recent report have shown honey being a chemotherapeutic mediator which display high toxicity to tumour and cancer cells (Fukuda *et al.*, 2011). A matter of fact, the healing properties of honey is cited in the Holy Quran, Surat An-Nahl, verses 68 and 69.

Aside from honey produced by the well-known honey bees from the genus of *Apis mellifera*, there are also honey produced by the other types of the bee namely stingless bee. Both bees originates from the same family of Apidae, however they were differentiated into a different clade of subfamily, Apinae for sting bees and Meliponinae for stingless bees (Winston and Michener, 1977). Interestingly, stingless bee honey is claimed to be twice nutritious than other honey varieties. This is the consequence of the high number of reports from several continents citing stingless bee honey being beneficial in curing numerous health complications, including throat inflammation, gastritis, eye cataracts, post birth-recovery and etc. (Apiterapia, Ambiental and Andes, 2001; Borsato *et al.* 2014). In Malaysia, honey produced from the foraging activity of the stingless bee *Heterotrigona itama* or natively known as '*Kelulut'* is highly valued by the consumers as a food dietary complementary. The *H. itama* bee is among the most domesticated stingless bees in bee farming industry

(Meliponiculture), also known as a substantially growing industry in Malaysia (Jalil, 2014; Kelly *et al.,* 2014). *H. itama* bees store their honey in clusters of small resin pots enclosed by propolis and wax, unlike the hexagonal honey combs in *Apis* bee. The honey of the *H. itama* bee has a distinctive sweetness combining with a strong sour and acidic taste, and is characteristically more watery (Alves *et al.,* 2005). This type of bee produce considerably lesser honey than that of *Apis mellifera* (Carvalho *et al.,* 2005). In addition, the Malaysian bee farmers prefer to rear *H. itama* bees as such species are highly sustainable, less susceptible to seasonal changes and more proficient to survive extreme environment (Kelly *et al.,* 2014). It is also worth to mention here, that most of the Malaysian stingless bee honey in the market originates from *H. itama* bees. Prices of *H. itama* honey may reach up to a premium price of US100 per kg (RM400 per kg), costing almost twice as much as honey produced by the *Apis* bees (Kelly *et al.,* 2014).

Stingless bees incorporate some healthy symbionts microorganisms such as lactic acid bacteria (LAB) present in their gastrointestinal tract into the collected honey. LABs are among the beneficial microorganisms found in this honey that acts as probiotic when ingested. Certain *Lactobacillus* spp. have been shown to demonstrate good antimicrobial activity against both Gram negative and Gram positive pathogenic bacteria, as well as other spoilage bacteria (Audisio *et al.,* 2011; Aween *et al.,* 2012; Chua *et al.,* 2013; Tajabadi *et al.,* 2011). This LAB is also identified in the crop of adult stingless bee honey (Rokop, Horton and Newton, 2015). Likewise, *Fructobacillus* and *Lactobacillus* are among the bacterial species that colonize brood cells, “bee bread” (processed pollen) and nectar. (Rokop *et al.,* 2015). The incorporation of LAB as probiotics in foods has been regarded as one of the most progressive treatment possibilities without the use of drugs (Mudroňová *et al.,* 2011). A matter of fact, consumption of LAB has been proven useful in the fight against known pathogenic food-borne bacteria viz. *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* (Malaysian Intensive Care, 2012; Hughes *et al.,* 2005). Good antibacterial activity against multi-drug resistance bacteria (MDR) (Aljadi and Mohd Yusoff, 2003; Pedro and De Camargo, 2013), for instance, methicillin-resistant *Staphylococcus aureus* (MRSA) and vanomycin-sensitive *Enterococcus faecalis* (Pimentel *et al.,* 2013; Nishio *et al.,* 2016) have also been indicated. Indeed, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* are among the most frequently reported healthcare-associated pathogens in Malaysia (Malaysian Society of Intensive Care, 2012; Hughes *et al.,* 2005).

The many benefits of *H. itama* honey on human health are also associated with the presence of certain metabolites and compounds for instance, organics acid and bacteriocins (Rokop *et al.*, 2015). These beneficial compounds exist in honey as the by-product of biological activities of LAB (Aween *et al.*, 2012; Lee *et al.*, 2008) which are naturally present in this premium food. The hive of the stingless bee is home to a diverse array of microbes. As a consequence, social transmission and inoculation of the bee microbiome is a part of a dynamic process that contribute to microbial succession in the gut of bees (Anderson *et al.*, 2013; Kwong and Moran, 2016). As indicated in the literature, microbiome in honey can vary from one region to another (Grubbs *et al.*, 2015), so would the signature bioactive compounds and their corresponding bioactivity. There is a possibility that the differences in bioactivity of the collected *H. itama* honey is due to the strain-specific LABs, present in the samples. So far, much of the reports on the profile of stingless bee honey have originated from different regions of the globe such as that from the South Americas (Anderson *et al.,* 2013; Martinson *et al.,* 2011; Vásquez *et al.,* 2012) and the warmer parts of Europe (Escurede *et al.*, 2013; Killer *et al.*, 2014; Vojvodic *et al.,* 2013). Thus, the lack of comprehensive knowledge or reports on the microbiome and the kinds of bioactive constituents in local Malaysian *H. itama* honey would merit an interesting study and the information may prove useful in promoting this food commodity.

1.2 Problem Statement

Despite extensive global studies on lactic acid bacteria (LAB), data on LAB native to the raw Malaysian *H. itama* honey is sparse. Moreover, there is a huge demand for novel antibacterial agents effective against pathogenic bacteria resistant to the current antibiotics. This is because the issue of multi-drug resistant bacteria is on the rise. A reliable key resolution to this problem could be the use of natural compounds in honey to overcome the problem. Hence, the present study aimed in isolating, identifying LAB and assessing the physicochemical properties of *H. itama* honey, as well as tested for antibacterial activities against pathogenic bacteria.

This study also attempted to explore the changes in the nutritional properties of *H. itama* honey in relation to time by assessing the changes in certain physicochemical and bioactivity aspects. This is an important issue as most honey is not consumed instantly after harvested, especially if it is bought in a supermarket and, not directly from a beekeeper. It is hypothesized that the LAB may prove useful in maintaining the healthy bacterial population of the human gut to enable promotion of good health. Also, the study believes the nutritional of the honey would change over time in relation to changes in LAB population in the honey.

1.3 Research Objectives

This research evaluated stingless bee, *H. itama* honey from four different localities in Peninsular Malaysia. Hence, this study aims to achieve following three objectives:

1. To isolate, identify and assess the antibacterial activity of LAB species present in the Malaysian *H. itama* honey
2. To profile the physicochemical and bioactivity properties of *H. itama* honey
3. To carry out a time-course assessment on the physicochemical and bioactivity aspects of *H. itama* honey

1.4 Scopes of Study

Firstly, the study isolates four strains of lactic acid bacteria (LAB) from four fresh *H. itama* honey samples collected from four different localities in Peninsular Malaysian. The isolated LAB strains were initially subjected to catalase for confirming their characteristic as LAB and further characterized for gram staining and scanning electron microscopy (SEM). Molecular identification was done by DNA extraction, followed by amplification and analysis of the coding region of the 16S sub-unit of the bacterial ribosomes utilising universal forward and reverse primers. Phylogenetic analysis was constructed by using MEGA6 software to determine the closeness of isolated LAB to the existing LAB strains from the gene bank databases. The isolated LABs were evaluated on their ability to utilize different types of substrates within the carbohydrate's family using the API 50 CHL Kit. Following that, the antibacterial activities of each LAB strains were evaluated against four different clinically isolated pathogenic bacteria.

The honey samples were then characterized for their physical properties such as pH, moisture content, colour analysis and colour intensity. Subsequently, they were quantified for sugar content, protein content, hydromethylfurfural (HMF) content, total phenolic content (TPC) and total flavonoid content (TFC). *H. itama* honey samples were then assayed for bioactivity. Two colourimetric assays; DPPH free radical scavenging assay and ferric reducing antioxidant power (FRAP) were used as antioxidant capacity determinant. The antibacterial activities were evaluated using agar-well diffusion method and micro-dilution assay utilising resazurin as the effective growth indicator. Next, scanning electron microscopic (SEM) analysis were performed to observe the pathogenic bacterial morphological alteration caused by *H. itama* honey at inhibitory (MIC) and bactericidal concentrations (MBC), and the results were compared with multi-spectrum antibiotic, streptomycin.

Finally, this study observed the changes in physicochemical and bioactivity of *H. itama* honey during one-month storage. LAB count and physicochemical properties such as pH, moisture content and total phenolic content and total flavonoid content, DPPH radical scavenging activity were examined weekly to examine any significant changes over a storage time.

1.5 Significance of Study

The information gathered in this study may be used to enhance the nutritional identity for the Malaysian stingless bee honey, such as for product labelling and marketing along with the possibility of promoting a production chain for these native bee products. Beyond the health benefits of this honey, discovery and application of healthy microorganism may facilitate development of other biotechnological products and consequently, improving human lifestyle and human survival.

Most importantly, antibacterial studies against representative Gram-positive and Gram-negative bacteria may develop a new therapeutic development to combat MDR bacterial infection using *H. itama* honey in combination with the existing antibiotics. Correspondingly, data of time-course profiling may contribute to the body of knowledge with respect to the microbiome profile to impact changes in the physicochemical and nutritional aspects of stored *H. itama* honey.

REFERENCES

Ahmad, I., Jimenez, H., Yaacob, N. S., & Yusuf, N. (2012). Tualang honey protects keratinocytes from ultraviolet radiation-induced inflammation and DNA damage. *Photochemistry and Photobiology*, *88*(5), 1198–1204.

Ahmer, B. M. M. (2004). Cell-to-cell signalling in *Escherichia coli* and *Salmonella enterica*. *Molecular Microbiology*, *52*(4), 933–945.

Aizenberg-Gershtein, Y., Izhaki, I., & Halpern, M. (2013). Do Honeybees Shape the Bacterial Community Composition in Floral Nectar? *PLOS ONE*, *8*(7), e67556. doi.org/10.1371/journal.pone.0067556

Al-Waili, N. S., & Haq, A. (2004). Effect of honey on antibody production against thymus-dependent and thymus-independent antigens in primary and secondary immune responses. *Journal of Medicinal Food*, *7*(4), 491–494.

Aljadi, A. M., & Mohd Yusoff, K. (2003). Isolation and identification of phenolic acids in Malaysian honey with antibacterial properties. *Turkish Journal of Medical Science*, *33*(4), 229-236.

Allen, K. L., Hutchinson, G., & Molan, P. C. (2000). The potential for using honey to treat wounds infected with MRSA and VRE. In *First World Wound Healing Congress* (pp. 10–13).

Allen, K. L., Molan, P. C., & Reid, G. M. (1991). A survey of the antibacterial activity of some New Zealand honeys. *Journal of Pharmacy Pharmacology*, *43*. https://doi.org/10.1111/j.2042-7158.1991.tb03186.x

Alves, R. M. de O., Carvalho, C. A. L. de, Souza, B. de A., Sodré, G. da S., & Marchini, L. C. (2005). Physico-chemical characteristics of honey samples of stingless bee Melipona mandacaia Smith (Hymenoptera: Apidae). *Food Science and Technology (Campinas)*, *25*(4), 644–650.

Anderson, K. E., Sheehan, T. H., Mott, B. M., Maes, P., Snyder, L., Schwan, M. R., … Corby-Harris, V. (2013). Microbial Ecology of the Hive and Pollination Landscape: Bacterial Associates from Floral Nectar, the Alimentary Tract and Stored Food of Honey Bees (*Apis mellifera*). *PLOS ONE*, *8*(12), e83125.

Angmo, K., Kumari, A., & Bhalla, T. C. (2016). Antagonistic activities of lactic acid bacteria from fermented foods and beverage of Ladakh against Yersinia enterocolitica in refrigerated meat. *Food Bioscience*, *13*, 26–31.

Apiterapia, P. V., Ambiental, V., & Andes, L. (2001). Effect of Stingless Bee Honey in Selenite Cataracts. *Apiacta*, *3*, 37–40.

Aplevicz, K. S., Mazo, J. Z., Ilha, E. C., & Dinon, A. Z. (2014). Isolation and characterization of lactic acid bacteria and yeasts from the Brazilian grape sourdough. *Brazilian Journal of Pharmaceutical Sciences*, *50*(2), 321–327.

Askar, A. (1984). Flavour changes during production and storage of fruit juices. *Fluessiges Obst*, *51*, 564–569.

Audisio, M. C., Torres, J., & Sabate, D. C. (2011). Properties of different lactic acid bacteria isolated from *Apis mellifera* L . bee-gut, *Micrbiology Research, 166*. https://doi.org/10.1016/j.micres.2010.01.003

Aween, M. M., Hassan, Z., Muhialdin, B. J., Noor, H. M., & Eljamel, Y. A. (2012). Evaluation on antibacterial activity of *Lactobacillus acidophilus* strains isolated from honey. *American Journal of Applied Sciences*, *9*(6), 807.

Bakar, M. F. A., Sanusi, S. B., Bakar, F. I. A., Cong, O. J., & Mian, Z. (2017). Physicochemical and Antioxidant Potential of Raw Unprocessed Honey From Malaysian Stingless Bees. *Pakistan Journal of Nutrition*, *16*(11), 888–894.

Baroni, M. V, Chiabrando, G. A., Costa, C., & Wunderlin, D. A. (2002). Assessment of the floral origin of honey by SDS-page immunoblot techniques. *Journal of Agricultural and Food Chemistry*, *50*(6), 1362–1367.

Bath, P. K., & Singh, N. (1999). A comparison between Helianthus annuus and Eucalyptus lanceolatus honey. *Food Chemistry*, *67*(4), 389–397.

Belitz, H. D., & Grosch, W. (1999). Food chemistry. 2nd. New York: Springer.

Belitz, I. H.-D., & Grosch, I. W. (1999). Aroma substances. In *Food chemistry* (pp. 319–377). Springer.

Benzie, I. F. F., & Strain, J. J. (1999). [2] Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology*, *299*, 15–27.

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

Berge, A. C., & Wierup, M. (2012). Nutritional strategies to combat Salmonella in mono-gastric food animal production. *Animal*, *6*(4), 557–564.

Bijlsma, L., de Bruijn, L. L. M., Martens, E. P., & Sommeijer, M. J. (2006). Water content of stingless bee honeys (Apidae, Meliponini): interspecific variation and comparison with honey of *Apis mellifera*. *Apidologie*, *37*(4), 480–486.

Biluca, F. C., Braghini, F., Gonzaga, L. V., Costa, A. C. O., & Fett, R. (2016). Physicochemical profiles, minerals and bioactive compounds of stingless bee honey (Meliponinae). *Journal of Food Composition and Analysis*, *50*, 61–69.

Bogdanov, S., Martin, P., & Lullmann, C. (2002). Harmonised methods of the international honey commission. *Swiss Bee Research Centre, FAM, Liebefeld*.

Borsato, D. M., Prudente, A. S., Döll-Boscardin, P. M., Borsato, A. V., Luz, C. F. P., Maia, B. H. L. N. S., … Miguel, O. G. (2014). Topical Anti-Inflammatory Activity of a Monofloral Honey of *Mimosa scabrella* Provided by *Melipona marginata* During Winter in Southern Brazil. *Journal of Medicinal Food*, *17*(7), 817–825. https://doi.org/10.1089/jmf.2013.0024

Brudzynski, K., Miotto, D., Kim, L., Sjaarda, C., Maldonado-Alvarez, L., & Fukś, H. (2017). Active macromolecules of honey form colloidal particles essential for honey antibacterial activity and hydrogen peroxide production. *Scientific Reports*, *7*(1), 7637.

Butler, È., Alsterfjord, M., Olofsson, T. C., Karlsson, C., Malmström, J., & Vásquez, A. (2013). Proteins of novel lactic acid bacteria from *Apis mellifera* mellifera: an insight into the production of known extra-cellular proteins during microbial stress. *BMC Microbiology*, *13*(1), 235.

Care, M. S. of I. (2012). *Guide to Antimicrobial Therapy in the Adult ICU 2012*. Malaysian Society of Intensive Care (MSIC). Retrieved from https://books.google.com.my/books?id=x_cfnwEACAAJ

Castro-Vázquez, L., Díaz-Maroto, M. C., González-Viñas, M. A., De La Fuente, E., & Pérez-Coello, M. S. (2008). Influence of storage conditions on chemical composition and sensory properties of citrus honey. *Journal of Agricultural and Food Chemistry*, *56*(6), 1999–2006.

Cervantes, M. A. R., Novelo, S. A. G., & Duch, E. S. (2000). Effect of the temporary thermic treatment of honey on variation of the quality of the same during storage. *Apiacta*, *35*, 4.

Chang, C.-C., Yang, M.-H., Wen, H.-M., & Chern, J.-C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods.

Journal of Food and Drug Analysis, *10*(3).

Chen, H., & Hoover, D. G. (2003). Bacteriocins and their food applications. *Comprehensive Reviews in Food Science and Food Safety*, *2*(3), 82–100.

Chua, L. S., Abdul-Rahaman, N.-L., Sarmidi, M. R., & Aziz, R. (2012). Multi-elemental composition and physical properties of honey samples from Malaysia. *Food Chemistry*, *135*(3), 880–887.

Chua, L. S., Lee, J. Y., & Chan, G. F. (2013). Honey protein extraction and determination by mass spectrometry. *Analytical and Bioanalytical Chemistry*, *405*(10), 3063–3074. https://doi.org/10.1007/s00216-012-6630-2

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

Chuttong, B., Chanbang, Y., Sringarm, K., & Burgett, M. (2016). Physicochemical profiles of stingless bee (Apidae: Meliponini) honey from South East Asia (Thailand). *Food Chemistry*, *192*, 149–155.

Cimo, G., & Conte, P. (2015). Conformational redistribution of honey components following different storage conditions. *International Journal of Spectroscopy*, *2015*.

Corcoran, B. M., Stanton, C., Fitzgerald, G., & Ross, R. P. (2008). Life under stress: the probiotic stress response and how it may be manipulated. *Current Pharmaceutical Design*, *14*(14), 1382–1399.

D'Errico, M., Lemma, T., Calcagnile, A., De Santis, L. P., & Dogliotti, E. (2007). Cell type and DNA damage specific response of human skin cells to environmental agents. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *614*(1), 37–47.

da Silva, I. A. A., da Silva, T. M. S., Camara, C. A., Queiroz, N., Magnani, M., de Novais, J. S., … de Souza, A. G. (2013). Phenolic profile, antioxidant activity and palynological analysis of stingless bee honey from Amazonas, Northern Brazil. *Food Chemistry*, *141*(4), 3552–3558.

de Carvalho, C. A. L., de Almeida Souza, B., da Silva Sodré, G., Marchini, L. C., & de Oliveira Alves, R. M. (2005). *Mel de abelhas sem ferrão: contribuição para a caracterização físico-química*. Insecta-Núcleo de Estudos dos Insetos.

De Man, J. C., Rogosa, M., & Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology*, *23*(1), 130–135.

https://doi.org/10.1111/j.1365-2672.1960.tb00188.x

de Queiroz Pimentel, R. B., da Costa, C. A., Albuquerque, P. M., & Junior, S. D. (2013). Antimicrobial activity and rutin identification of honey produced by the stingless bee Melipona compressipes manaosensis and commercial honey. *BMC Complementary and Alternative Medicine*, *13*(1), 151.

Deegan, L. H., Cotter, P. D., Hill, C., & Ross, P. (2006). Bacteriocins: biological tools for bio-preservation and shelf-life extension. *International Dairy Journal*, *16*(9), 1058–1071.

Díez, M. J., Andrés, C., & Terrab, A. (2004). Physicochemical parameters and pollen analysis of Moroccan honeydew honeys. *International Journal of Food Science & Technology*, *39*(2), 167–176.

Drummond, A. J., & Waigh, R. D. (2000). The development of microbiological methods for phytochemical screening. *Recent Research Developments in Phytochemistry*, *4*, 143–152.

Dutra, R. P., Abreu, B. V. de B., Cunha, M. S., Batista, M. C. A., Torres, L. M. B., Nascimento, F. R. F., … Guerra, R. N. M. (2014). Phenolic acids, hydrolyzable tannins, and antioxidant activity of geopropolis from the stingless bee Melipona fasciculata Smith. *Journal of Agricultural and Food Chemistry*, *62*(12), 2549–2557.

Endo, A., Irisawa, T., Futagawa-Endo, Y., Takano, K., du Toit, M., Okada, S., & Dicks, L. M. T. (2012). Characterization and emended description of Lactobacillus kunkeei as a fructophilic lactic acid bacterium. *International Journal of Systematic and Evolutionary Microbiology*, *62*(Pt 3), 500–504. https://doi.org/10.1099/ijs.0.031054-0

Endo, A., & Okada, S. (2008). Reclassification of the genus Leuconostoc and proposals of Fructobacillus fructosus gen. nov., comb. nov., Fructobacillus durionis comb. nov., Fructobacillus ficulneus comb. nov. and Fructobacillus pseudoficulneus comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, *58*(9), 2195–2205.

Endo, A., & Salminen, S. (2013). Honeybees and beehives are rich sources for fructophilic lactic acid bacteria. *Systematic and Applied Microbiology*, *36*(6), 444–448.

Escuredo, O., Míguez, M., Fernández-González, M., & Seijo, M. C. (2013). Nutritional value and antioxidant activity of honeys produced in a European

Atlantic area. *Food Chemistry*, *138*(2), 851–856.

Escuredo, O., Seijo, M. C., & Fernández-González, M. (2011). Descriptive analysis of Rubus honey from the north-west of Spain. *International Journal of Food Science & Technology*, *46*(11), 2329–2336.

Esti, M., Panfili, G., Marconi, E., & Trivisonno, M. C. (1997). Valorization of the honeys from the Molise region through physico-chemical, organoleptic and nutritional assessment. *Food Chemistry*, *58*(1), 125–128.

Fallico, B., Zappala, M., Arena, E., & Verzera, A. (2004). Effects of conditioning on HMF content in unifloral honeys. *Food Chemistry*, *85*(2), 305–313.

Ferreira, I. C. F. R., Aires, E., Barreira, J. C. M., & Estevinho, L. M. (2009). Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry*, *114*(4), 1438–1443.

Forsgren, E., Olofsson, T. C., Vásquez, A., & Fries, I. (2010). Novel lactic acid bacteria inhibiting Paenibacillus larvae in honey bee larvae. *Apidologie*, *41*(1), 99–108.

Fukuda, M., Kobayashi, K., Hirono, Y., Miyagawa, M., Ishida, T., Ejiogu, E. C., … Takeuchi, M. (2011). Jungle honey enhances immune function and antitumor activity. *Evidence-Based Complementary and Alternative Medicine*, *2011*.

Gheldof, N., & Engeseth, N. J. (2002). Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. *Journal of Agricultural and Food Chemistry*, *50*(10), 3050–3055.

Grubbs, K. J., Scott, J. J., Budsberg, K. J., Read, H., Balser, T. C., & Currie, C. R. (2015). Unique honey bee (*Apis mellifera*) hive component-based communities as detected by a hybrid of phospholipid fatty-acid and fatty-acid methyl ester analyses. *PLoS ONE*, *10*(4), e0121697. https://doi.org/10.1371/journal.pone.0121697

Gulati, R., & Kumari, B. (2005). Chemical composition of unifloral, stored and commercial *Apis mellifera* L. honeys. *Journal of Food Science and Technology-Mysore*, *42*(6), 492–495.

He, H., Chen, Y., Zhang, Y., & Wei, C. (2011). Bacteria associated with gut lumen of Camponotus japonicus Mayr. *Environmental Entomology*, *40*(6), 1405–1409.

Henriques, A. F., Jenkins, R. E., Burton, N. F., & Cooper, R. A. (2011). The effect of manuka honey on the structure of Pseudomonas aeruginosa. *European Journal* *of Clinical Microbiology & Infectious Diseases*, *30*(2), 167–171.

Holt, J. G., Williams, S. T., & Holt. (1989). *Bergey's manual of systematic bacteriology, Vol. 4*. Lippincott Williams & Wilkins.

Hughes, A. J., Ariffin, N., Tan, L. H., Abdul Muluk, H., & Hashim, S. (2005). Prevalence of Nosocomial infection and antibiotic use at a university medical center in Malaysia. *Infection Control Hospital Epidemiology*, *26*. https://doi.org/10.1086/502494

Ismail, N. I., Abdul Kadir, M. R., Mahmood, N. H., Singh, O. P., Iqbal, N., & Zulkifli, R. M. (2016). Apini and Meliponini foraging activities influence the phenolic content of different types of Malaysian honey. *Journal of Apicultural Research*, *55*(2), 137–150.

Iurlina, M. O., Saiz, A. I., Fritz, R., & Manrique, G. D. (2009). Major flavonoids of Argentinean honeys. Optimisation of the extraction method and analysis of their content in relationship to the geographical source of honeys. *Food Chemistry*, *115*(3), 1141–1149.

Jalil, A. H. (2014). *Beescape for Meliponines: Conservation of Indo-Malayan Stingless Bees*. Partridge Publishing Singapore.

Jenkins, R., Burton, N., & Cooper, R. (2011). Manuka honey inhibits cell division in methicillin-resistant Staphylococcus aureus. *Journal of Antimicrobial Chemotherapy*, *66*(11), 2536–2542.

Jiménez, M., Mateo, J. J., Huerta, T., & Mateo, R. (1994). Influence of the storage conditions on some physicochemical and mycological parameters of honey. *Journal of the Science of Food and Agriculture*, *64*(1), 67–74.

Jones, J. C., Jones, J. C., Myerscough, M. R., Graham, S., & Oldroyd, B. P. (2014). Honey Bee Nest Thermoregulation : Diversity Promotes Stability, *402*(2004). https://doi.org/10.1126/science.1096340

Kakkar, S., & Bais, S. (2014). A review on protocatechuic acid and its pharmacological potential. *ISRN Pharmacology*, *2014*.

Kelly, N., Farisya, M. S. N., Kumara, T. K., & Marcela, P. (2014). Species Diversity and External Nest Characteristics of Stingless Bees in Meliponiculture. *Pertanika Journal of Tropical Agricultural Science*, *37*(3).

Kenji Nishio, E., Carolina Bodnar, G., Regina Eches Perugini, M., Cornélio Andrei, C., Aparecido Proni, E., Katsuko Takayama Kobayashi, R., & Nakazato, G. (2016). Antibacterial activity of honey from stingless bees *Scaptotrigona* *bipunctata* Lepeletier, 1836 and *S. postica* Latreille, 1807 (Hymenoptera: Apidae: Meliponinae) against methicillin-resistant *Staphylococcus aureus* (MRSA). *Journal of Apicultural Research*, *8839*, 1–9. https://doi.org/10.1080/00218839.2016.1162985

Khalil, M. I., Moniruzzaman, M., Boukraâ, L., Benhanifia, M., Islam, M. A., Islam, M. N., … Gan, S. H. (2012). Physicochemical and antioxidant properties of Algerian honey. *Molecules*, *17*(9), 11199–11215.

Khan, K. A., Ansari, M. J., Al-Ghamdi, A., Nuru, A., Harakeh, S., & Iqbal, J. (2017). Investigation of gut microbial communities associated with indigenous honey bee (*Apis mellifera* jemenitica) from two different eco-regions of Saudi Arabia. *Saudi Journal of Biological Sciences*.

Killer, J., Dubná, S., Sedláček, I., & Švec, P. (2014). Lactobacillus apis sp. nov., from the stomach of honeybees (*Apis mellifera*), having an in vitro inhibitory effect on the causative agents of American and European foulbrood. *International Journal of Systematic and Evolutionary Microbiology*. https://doi.org/10.1099/ijs.0.053033-0

Kim, W., Gilet, T., & Bush, J. W. M. (2011). Optimal concentrations in nectar feeding. *Proceedings of the National Academy of Sciences*.

Koch, H., & Schmid-Hempel, P. (2011). Bacterial communities in central European bumblebees: low diversity and high specificity. *Microbial Ecology*, *62*(1), 121–133.

Kwong, W. K., & Moran, N. A. (2016). Gut microbial communities of social bees. *Nature Reviews Microbiology*, *14*(6), 374–384. https://doi.org/10.1038/nrmicro.2016.43

Lee, H., Churey, J. J., & Worobo, R. W. (2008). Antimicrobial activity of bacterial isolates from different floral sources of honey. *International Journal of Food Microbiology*, *126*(1), 240–244.

Lemos, M. S., Venturieri, G. C., Dantas Filho, H. A., & Dantas, K. G. F. (2017). Evaluation of the physicochemical parameters and inorganic constituents of honeys from the Amazon region. *Journal of Apicultural Research*, 1–10.

Ludwig, W., Strunk, O., Klugbauer, S., Klugbauer, N., Weizenegger, M., Neumaier, J., … Schleifer, K. H. (1998). Bacterial phylogeny based on comparative sequence analysis. *Electrophoresis*, *19*, 554–568. https://doi.org/10.1002/elps.1150190416

Manyi-Loh, C. E., Clarke, A. M., & Ndip, R. N. (2011). An overview of honey: therapeutic properties and contribution in nutrition and human health. *African Journal of Microbiology Research*, *5*.

Martinson, V. G., Danforth, B. N., Minckley, R. L., Rueppell, O., Tingek, S., & Moran, N. A. (2011). A simple and distinctive microbiota associated with honey bees and bumble bees. *Molecular Ecology*, *20*(3), 619–628.

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

Mei, S. J., Nordin, M. S. M., & Norrakiah, A. S. (2010). Fructooligosaccharides in honey and effects of honey on growth of *Bifidobacterium longum* BB 536. *International Food Research Journal*, *17*(3), 557–561.

Mercês, M. D., Peralta, E. D., Uetanabaro, A. P. T., & Lucchese, A. M. (2013). Antimicrobial activity of honey from five species of Brazilian stingless bees. *Ciência Rural*, *43*(4), 672–675.

Miliauskas, G., Venskutonis, P. R., & Van Beek, T. A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry*, *85*(2), 231–237.

Mills, S., Stanton, C., Fitzgerald, G. F., & Ross, Rp. (2011). Enhancing the stress responses of probiotics for a lifestyle from gut to product and back again. In *Microbial cell factories* (Vol. 10, p. S19). BioMed Central.

Miyauchi, E., O'Callaghan, J., Buttó, L. F., Hurley, G., Melgar, S., Tanabe, S., … O'Toole, P. W. (2012). Mechanism of protection of transepithelial barrier function by *Lactobacillus salivarius*: strain dependence and attenuation by bacteriocin production. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *303*(9), G1029–G1041.

Molan, P. C. (1992). The antibacterial activity of honey 1. The nature of antibacterial activity. *Bee World*, *73*. https://doi.org/10.1080/0005772X.1992.11099109

Moniruzzaman, M., Sulaiman, S. A., Khalil, M. I., & Gan, S. H. (2013). Evaluation of physicochemical and antioxidant properties of sourwood and other Malaysian honeys: a comparison with manuka honey. *Chemistry Central Journal*, *7*(1), 138.

Moo-Huchin, V. M., Gonzalez-Aguilar, G. A., Lira-Maas, J. D., Perez-Pacheco, E., Estrada-León, R., Moo-Huchin, M. I., & Sauri-Duch, E. (2015). Physicochemical Properties of Melipona beecheii Honey of the Yucatan Peninsula. *Journal of Food Research*, *4*(5), 25.

Mudroňová, D., Toporčák, J., Nemcová, R., Gancarčíková, S., Hajdučková, V., & Rumanovská, K. (2011). Lactobacillus sp. as a potential probiotic for the prevention of Paenibacillus larvae infection in honey bees. *Journal of Apicultural Research*, *50*(4), 323–324.

Neveling, D. P., Endo, A., & Dicks, L. M. T. (2012). Fructophilic Lactobacillus kunkeei and Lactobacillus brevis isolated from fresh flowers, bees and bee-hives. *Current Microbiology, 65*(5), 507–515. https://doi.org/10.1007/s00284-012-0186-4

Norowi, M. H., Sajap, A. S., Rosliza, J., Fahimie, M. J., & Suri, R. (2010). Conservation and sustainable utilization of stingless bees for pollination services in agricultural ecosystems in Malaysia. International Seminar on Enhancement of Functional Biodiversity Relevent to Sustainable Food Production in ASPAC.

Oddo, L. P., Heard, T. A., Rodríguez-Malaver, A., Pérez, R. A., Fernández-Muiño, M., Sancho, M. T., … Vit, P. (2008). Composition and antioxidant activity of Trigona carbonaria honey from Australia. *Journal of Medicinal Food*, *11*(4), 789–794.

Olofsson, T. C., & Vásquez, A. (2008). Detection and Identification of a Novel Lactic Acid Bacterial Flora Within the Honey Stomach of the Honeybee *Apis mellifera*. *Current Microbiology*, *57*(4), 356–363. https://doi.org/10.1007/s00284-008-9202-0

Olofsson, T. C., & Vásquez, A. (2009). Phylogenetic comparison of bacteria isolated from the honey stomachs of honey bees *Apis mellifera* and bumble bees Bombus spp. *Journal of Apicultural Research*, *48*(4), 233–237. https://doi.org/10.3896/IBRA.1.48.4.02

Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J. A., & Deemer, E. K. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. *Journal of Agricultural and Food Chemistry*, *50*(11), 3122–3128.

Pedro, S. R. M., & De Camargo, J. M. F. (2013). Stingless bees from Venezuela. In

Pot-Honey: A Legacy of Stingless Bees (Vol. 9781461449, pp. 73–86). Springer. https://doi.org/10.1007/978-1-4614-4960-7_4

Ramón-Sierra, J. M., Ruiz-Ruiz, J. C., & de la Luz Ortiz-Vázquez, E. (2015). Electrophoresis characterisation of protein as a method to establish the entomological origin of stingless bee honeys. *Food Chemistry*, *183*, 43–48.

Riazi, A., & Ziar, H. (2012). Effect of honey and starter culture on growth, acidification, sensory properties and bifidobacteria cell counts in fermented skimmed milk. *African Journal of Microbiology Research*, *6*(3), 486–498.

Roberts, A. E. L., Maddocks, S. E., & Cooper, R. A. (2014). Manuka honey reduces the motility of Pseudomonas aeruginosa by suppression of flagella-associated genes. *Journal of Antimicrobial Chemotherapy*, *70*(3), 716–725.

Rogosa, M., Mitchell, J. A., & Wiseman, R. F. (1951). A selective medium for the isolation and enumeration of oral lactobacilli. *Journal of Dental Research*, *30*(5), 682–689.

Rokop, Z. P., Horton, M. A., & Newton, I. L. G. (2015). Interactions between cooccurring lactic acid bacteria in honey bee hives. *Applied and Environmental Microbiology*, *81*(20), 7261–7270.

Saxena, S., Gautam, S., & Sharma, A. (2010). Physical, biochemical and antioxidant properties of some Indian honeys. *Food Chemistry*, *118*(2), 391–397.

Sechrist, E. L. (1925). *The color grading of honey*. US Department of Agriculture.

Sesta, G., & Lusco, L. (2008). Refractometric determination of water content in royal jelly. *Apidologie*, *39*(2), 225–232.

Setlow, P. (2006). Spores of Bacillus subtilis: their resistance to and killing by radiation, heat and chemicals. *Journal of Applied Microbiology*, *101*(3), 514–525.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in Enzymology*.

Somasundram, C., Razali, Z., & Santhirasegaram, V. (2016). A Review on Organic Food Production in Malaysia. *Horticulturae*, *2*(3), 12.

Southwick, E. E., Loper, G. M., & Sadwick, S. E. (1981). Nectar production, composition, energetics and pollinator attractiveness in spring flowers of western New York. *American Journal of Botany*, *68*(7), 994–1002.

Souza, B. A., Roubik, D. W., Barth, O. M., Heard, T. A., Enriquez, E., Carvalho, C.,

… Persano-Oddo, L. (2006). Composition of stingless bee honey: setting quality standards. *Interciencia*, *31*(12).

Spano, N., Casula, L., Panzanelli, A., Pilo, M. I., Piu, P. C., Scanu, R., … Sanna, G. (2006). An RP-HPLC determination of 5-hydroxymethylfurfural in honey: The case of strawberry tree honey. *Talanta*, *68*(4), 1390–1395.

Tajabadi, N., Mardan, M., Manap, M. A., Shuhaimi, M., Meimandipour, A., Nateghi, L., … Shuhaimi, M. (2011). Detection and identification of Lactobacillus bacteria found in the honey stomach of the giant honeybee Apis dorsata.

Takahama, U., Hirota, S., Nishioka, T., & Oniki, T. (2003). Human salivary peroxidase-catalyzed oxidation of nitrite and nitration of salivary components 4-hydroxyphenylacetic acid and proteins. *Archives of Oral Biology*, *48*(10), 679–690.

Terrab, A., Recamales, A. F., Hernanz, D., & Heredia, F. J. (2004). Characterisation of Spanish thyme honeys by their physicochemical characteristics and mineral contents. *Food Chemistry*, *88*(4), 537–542.

Thaochan, N., Drew, R. A. I., Hughes, J. M., Vijaysegaran, S., & Chinajariyawong, A. (2010). Alimentary tract bacteria isolated and identified with API-20E and molecular cloning techniques from Australian tropical fruit flies, Bactrocera cacuminata and B. tryoni. *Journal of Insect Science*, *10*(1), 131.

Tornuk, F., Karaman, S., Ozturk, I., Toker, O. S., Tastemur, B., Sagdic, O., … Kayacier, A. (2013). Quality characterization of artisanal and retail Turkish blossom honeys: Determination of physicochemical, microbiological, bioactive properties and aroma profile. *Industrial Crops and Products*, *46*, 124–131.

Vásquez, A., Forsgren, E., Fries, I., Paxton, R. J., Flaberg, E., Szekely, L., & Olofsson, T. C. (2012). Symbionts as Major Modulators of Insect Health: Lactic Acid Bacteria and Honeybees. *PLOS ONE*, *7*(3), e33188. Retrieved from https://doi.org/10.1371/journal.pone.0033188

Veress, A., Kömüves, J., Wilk, T., Zajácz, E., Kerényi, Z., Kocsis, R., … Papp, P. (2016). Analysis of bacteria isolated from honey and honeybee stomach. *New Biotechnology*, (33), S175–S176.

Vit, P., Bogdanov, S., & Kilchenmann, V. (1994). Composition of Venezuelan honeys from stingless bees (Apidae: Meliponinae) and *Apis mellifera* L. *Apidologie*, *25*(3), 278–288.

Vit, P., Fernandez-Maeso, M. C., & Ortiz-Valbuena, A. (1998). Potential use of the three frequently occurring sugars in honey to predict stingless bee entomological origin. *Journal of Applied Entomology*, *122*(1-5), 5–8.

Vojvodic, S., Rehan, S. M., & Anderson, K. E. (2013). Microbial Gut Diversity of Africanized and European Honey Bee Larval Instars. *PLOS ONE*, *8*(8), e72106. Retrieved from https://doi.org/10.1371/journal.pone.0072106

Vrancken, G., Rimaux, T., Weckx, S., De Vuyst, L., & Leroy, F. (2009). Environmental pH determines citrulline and ornithine release through the arginine deiminase pathway in Lactobacillus fermentum IMDO 130101. *International Journal of Food Microbiology*, *135*(3), 216–222.

Weston, R. J. (2000). The contribution of catalase and other natural products to the antibacterial activity of honey: a review. *Food Chemistry*, *71*(2), 235–239.

White, J. W., & Doner, L. W. (1980). Honey composition and properties. *Beekeeping in the United States Agriculture Handbook*, *335*, 82–91.

White Jr, J. W. (1979). Spectrophotometric method for hydroxymethylfurfural in honey. *Journal-Association of Official Analytical Chemists*, *62*(3), 509–514.

Wijesinghe, W., & Jeon, Y.-J. (2011). Biological activities and potential cosmeceutical applications of bioactive components from brown seaweeds: a review. *Phytochemistry Reviews*, *10*(3), 431–443.

Winston, M. L., & Michener, C. D. (1977). Dual origin of highly social behavior among bees. *Proceedings of the National Academy of Sciences*, *74*(3), 1135–1137.

Won, S.-R., Lee, D.-C., Ko, S. H., Kim, J.-W., & Rhee, H.-I. (2008). Honey major protein characterization and its application to adulteration detection. *Food Research International*, *41*(10), 952–956.

Yaghoobi, N., Al-Waili, N., Ghayour-Mobarhan, M., Parizadeh, S. M. R., Abasalti, Z., Yaghoobi, Z., … Aghasizadeh, R. (2008). Natural honey and cardiovascular risk factors; effects on blood glucose, cholesterol, triacylglycerole, CRP, and body weight compared with sucrose. *The Scientific World Journal*, *8*, 463–469.

Zainol, M. I., Yusoff, K. M., Yasim, M., & Yusof, M. (2013). Antibacterial activity of selected Malaysian honey.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, *64*(4), 555–559.