



**UNIVERSITI PUTRA MALAYSIA**

**DEVELOPMENT OF GOOD HONEY INDEX FOR STINGLESS BEE  
HONEY BASED ON ITS COMPREHENSIVE PHYSICOCHEMICAL  
PROFILING**

**MUHAMMAD FAHDLI ABDUL RAHMAN**

**FBSB 2019 13**



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By

**MUHAMMAD FAHDLI BIN ABDUL RAHMAN**

**Thesis Submitted to School of Graduate Studies, Universiti Putra  
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Master of Science**

**November 2018**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in  
fulfilment of the requirement for the degree of Master of Science

## **DEVELOPMENT OF GOOD HONEY INDEX FOR STINGLESS BEE HONEY BASED ON ITS COMPREHENSIVE PHYSICOCHEMICAL PROFILING**

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**November 2018**

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Honey is the oldest natural and nutritious food that can support human health. Honey is very important in Malaysia as daily supplement food. Recently, the demand for stingless bee honey has increased due to its extraordinary benefits. Unfortunately, the honey naturally has limited production, and there is no strict procedure on how to sell good and authentic honey. Therefore, fake honey is widely sold in the marketplace to fulfil economic needs. This work focused on comprehensive profiling and the development of a good honey index (GHI) for stingless bee honey. The GHI composes three components; 1) the characteristic of honey, 2) the goodness of honey, and 3) the compliance to the Malaysian Standard (MS 2683:2017). Each component was constructed by several sub-components, and the data matrices were validated using statistical tools. 29 honey samples were tested, including 13 that were harvested from local bee farms, and 16 were bought from the local market. Investigation showed that chemical and elemental composition level is significantly different in most honey samples. The parameter for the characterization of comprehensive profiling such sugar and metal profiles was applied with the similarity distance. However, hierarchical cluster analysis (HCA) suggested that some composition patterns followed the bee species. The physicochemical properties of stingless bee honey was analyzed in the goodness of honey component includes metal daily consumption, water activity ( $a_w$ ), antimicrobial activity, total phenolic compound, DPPH and FRAP antioxidant activities. While under the compliance to Malaysian Standard comparisons of sugar; fructose and glucose, sucrose, maltose are made for the requirement of sugar level of stingless bee honey. Factorial analysis was carried out using the Kaiser-Meyer-Olkin (KMO) analysis in three components such as the characteristic of honey, the goodness of honey, and the compliance to Malaysian Standard for stingless bee that resulted in a KMO value of 0.661, 0.735 and 0.614 with all the component with  $p$ -value  $\leq 0.05$ . Power analysis was carried out to measure the adequacy of the sample size,

resulted in 96.4% of sampling power. The limit for GHI was set at 60, and 90% of raw honey samples passed the index threshold with the highest score of 89 from sample K91. For the commercial honey samples, only 50% passed the GHI threshold. In conclusion, a chemometric analysis of a complex characteristic of stingless bee honey has been demonstrated. Consequently, a newly developed GHI that can be used for the grading system to evaluate and qualify stingless bee honey quality for commercialization.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

## **PEMBANGUNAN INDEKS MADU BAGI MADU LEBAH KELULUT BERDASARKAN PROFIL FIZIKOKIMIA SECARA KOMPREHENSIF**

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Madu adalah makanan semula jadi dan berkhasiat yang tertua yang dapat membantu kesihatan manusia. Madu sangat penting di Malaysia sebagai makanan tambahan harian. Baru-baru ini, tuntutan mengenai madu lebah kelulut telah meningkat disebabkan oleh manfaatnya yang luar biasa. Namun, madu secara semulajadi mempunyai pengeluaran yang terhad dan tidak ada prosedur yang ketat tentang bagaimana untuk menjual madu yang baik dan asli. Oleh itu, madu palsu dijual secara meluas di pasaran untuk memenuhi keperluan ekonomi. Kerja-kerja ini memberi tumpuan kepada profil yang komprehensif dan pembangunan indeks madu yang baik (GHI) untuk madu lebah kelulut. GHI menyusun tiga komponen; 1) ciri-ciri madu, 2) kebaikan madu, dan 3) pematuhan kepada Standard Malaysia (MS 2683: 2017). Setiap komponen dibina oleh beberapa sub-komponen, dan matriks data telah disahkan menggunakan alat statistik. 29 sampel madu diuji, termasuk 13 yang dituai dari ladang lebah tempatan, dan 16 dibeli dari pasaran tempatan. Penyiasatan menunjukkan bahawa tahap komposisi kimia dan elemen sangat berbeza dalam kebanyakan sampel madu. Parameter untuk penyaringan profil ciri-ciri gula dan logam yang komprehensif digunakan dengan jarak persamaan. Walau bagaimanapun, analisis kluster hierarki (HCA) mencadangkan bahawa beberapa corak komposisi mengikuti spesies lebah. Ciri-ciri fizikokimia madu lebah kelulut dianalisis dalam kebaikan komponen madu termasuk pengambil kuantiti logam harian, aktiviti air ( $a_w$ ), aktiviti antimikrob, jumlah kompaun fenolik, DPPH dan FRAP aktiviti antioksidan. Dibawah pematuhan perbandingan kandungan gula Standard Malaysia; fruktosa dan glukosa, sukrosa, maltosa dibuat untuk keperluan tahap gula madu lebah kelulut. Analisis Factorial dilakukan menggunakan analisis Kaiser-Meyer-Olkin (KMO) dalam tiga komponen seperti ciri-ciri madu, kebaikan madu, dan pematuhan kepada Standard Malaysia untuk lebah kelulut, yang menghasilkan nilai KMO 0.661, 0.735 dan 0.614 dengan semua komponen dengan p-nilai  $\leq 0.05$ . Untuk mengukur kecukupan saiz sampel, analisis kuasa dijalankan, menghasilkan

99.9% kuasa pensampelan. Batasan untuk GHI ditetapkan pada 60, dan 90% sampel madu asli melebihi batasan indeks dengan tertinggi 89 dari sampel K91. Untuk sampel madu komersial, hanya 50% lulus batasan GHI. Sebagai kesimpulan, analisis chemometrik ciri-ciri kompleks madu lebah kelulut telah ditunjukkan. Ini mengakibatkan GHI yang baru dibangunkan yang boleh digunakan untuk sistem penggredan untuk menilai dan memenuhi syarat kualiti madu lebah kelulut untuk pengkomersialan.



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I certify that a Thesis Examination Committee has met on 23 November 2018 to conduct the final examination of Muhammad Fahdli bin Abdul Rahman on his thesis entitled "Development of Good Honey Index for Stingless Bee Honey Based on its Comprehensive Physicochemical Profiling" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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## TABLE OF CONTENTS

	<b>Page</b>
<b>ABSTRACT</b>	i
<b>ABSTRAK</b>	iii
<b>ACKNOWLEDGEMENTS</b>	v
<b>APPROVAL</b>	vi
<b>DECLARATION</b>	viii
<b>LIST OF TABLES</b>	xii
<b>LIST OF FIGURES</b>	xiii
<b>LIST OF APPENDICES</b>	xiv
<b>LIST OF ABBREVIATIONS</b>	xv
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
<b>2 LITERATURE REVIEW</b>	<b>3</b>
2.1 Stingless bee honey	3
2.2 Types of stingless bee honey	4
2.3 Properties of stingless bee honey	5
2.4 Carbohydrates analysis and profiling	6
2.5 Metal analysis and profiling	7
2.6 Phenolic compound in honey	8
2.7 Antioxidant properties in honey	9
2.8 Water activity ( $a_w$ ) in honey	9
2.9 Antimicrobial properties in honey	10
2.10 HPLC and ICP-MS instrument principle	11
2.11 Statistical analysis	12
<b>3 MATERIALS AND METHODS</b>	<b>13</b>
3.1 Material, equipment, and honey sample	13
3.1.1 Overview of the Good Honey Index strategy	14
3.1.2 Sample honey collection	14
3.2 The characteristic of honey	15
3.2.1 Determination of sugars compound	16
3.2.2 Determination of metals compound	17
3.2.3 Honey sugar distance (Euclidean distance)	17
3.2.4 Honey sugar distance (cosine distance)	18
3.2.5 Honey metal distance (cosine distance)	19
3.3 The goodness of honey	20
3.3.1 Metal daily consumption	20
3.3.2 Water activity ( $a_w$ )	21

	3.3.3	Antimicrobial activity	21
	3.3.4	Total phenolic compound (TPC)	21
	3.3.5	Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging activity	22
	3.3.6	Ferric Reducing Antioxidant Power (FRAP)	22
	3.4	Honey sugar compliance to Malaysian Standard (MS 2683:2017)	23
	3.5	Statistical analysis of GHI	23
<b>4</b>		<b>RESULTS AND DISCUSSION</b>	<b>25</b>
	4.1	Good Honey Index (GHI)	25
	4.2	The characteristic of honey	25
	4.2.1	Determination of sugars compound	26
	4.2.2	Determination of metals compound	29
	4.2.3	Honey sugar distance (Euclidean distance)	32
	4.2.4	Honey sugar distance (cosine distance)	35
	4.2.5	Honey metal distance (cosine distance)	38
	4.3	The goodness of honey	41
	4.3.1	Metal daily consumption	41
	4.3.2	Water activity ( $a_w$ )	44
	4.3.3	Antimicrobial activity	46
	4.3.4	Total phenolic compound	48
	4.3.5	DPPH antioxidant assay	50
	4.3.6	FRAP antioxidant assay	52
	4.4	Compliance with the Malaysian Standard (MS 2683:2017) in Good Honey Index	54
	4.4.1	Compliance to fructose and glucose MS 2683:2017	54
	4.4.2	Compliance to sucrose MS 2683:2017	55
	4.4.3	Compliance to maltose MS 2683:2017	56
	4.5	Statistical analysis of GHI	57
<b>5</b>		<b>CONCLUSION</b>	<b>61</b>
		<b>REFERENCES</b>	<b>62</b>
		<b>APPENDICES</b>	<b>69</b>
		<b>BIODATA OF STUDENT</b>	<b>77</b>

## LIST OF TABLES

<b>Table</b>		<b>Page</b>
1	Classification of stingless bee species	4
2	Quality characteristic requirement by MS (2683:2017)	7
3	Bacteria species in antimicrobial activity	10
4	List of chemicals	13
5	List of equipments	13
6	Honey samples label	15
7	Sugar honey profiles	27
8	Metal honey profiles	29
9	Number of honey samples of metal concentration compared to average of the raw honey samples	31
10	Metal dosage for daily intake	41
11	Mean values total percentage of metal concentration in honey samples	42
12	Mean values of water activity analysis of honey samples	44
13	Mean values of inhibition an antimicrobial activity of honey samples	46
14	Mean values of absorbance total phenolic compound analysis of honey samples	48
15	Mean values of absorbance DPPH activity 5% honey of honey samples	50
16	Mean values of absorbance FRAP antioxidant activity of honey samples	52
17 (a)	Score data and Good Honey Index profiles of 13 raw honey samples	58
17 (b)	Score data and Good Honey Index profiles of 16 commercial honey samples	59

## LIST OF FIGURES

Figure		Page
1	An overview the phase of GHI	14
2	Honey collection and harvested place	15
3	Sugar Euclidean distance of honey samples	33
4	Percentage of number honey samples of sugar Euclidean distance	34
5	Sugar cosine distance of honey samples	36
6	Percentage of number honey samples of sugar cosine distance	37
7	Metal cosine distance of honey samples	39
8	Percentage of number honey samples of metal cosine distance	40
9	Percentage of honey number against the amount daily consumption (%)	43
10	Percentage of honey number against the range of water activity in unit $a_w$	45
11	Percentage of number honey sample against the range of inhibition growth of bacteria with 100% honey unit as mm	47
12	Percentage number of honey against range total phenolic content (TPC) concentration in 5% honey	49
13	Percentage number of the honey sample against the range of DPPHs activity as a unit is (%)	51
14	Percentage number of the honey sample against range FRAPs concentration in 10% honey (mM).	53
15	Percentage number of the honey sample against range fructose+glucose (%) according to Malaysian Standard	54
16	Percentage number of the honey sample against range sucrose (%) according to Malaysian Standard	57
17	Percentage number of the honey sample against range maltose (%) according to Malaysian Standard	56
18	Summerize of quantitative data of Good Honey Index	57



## LIST OF APPENDICES

<b>Appendix</b>		<b>Page</b>
1	HPLC chromatogram of three sugars standard with 100 mM	69
2	Fructose standard curve	69
3	Glucose standard curve	70
4	Sucrose standard curve	70
5	Maltose standard curve	71
6	Gallic acid standard curve	71
7	FRAP standard curve	72
8	Threshold score and grading of Good Honey Index	72
9	Normality test by Gaussian distribution	73

## LIST OF ABBREVIATIONS

%	percent
µg	Microgram
µl	Microliter
°	Degree
°C	Degree celcius
$a_w$	Water activity
ACN	Acetonitrile
CA	Conformity analysis
Cu	Copper
Cd	Cadmium
Cr	Chromium
DAD	Diode array detector
dH <sub>2</sub> O	Deionized water
DPPH	2,2-diphenyl-1-picrylhydrazyl
FA	Factor analysis
Fe	Iron
FRAP	Ferric reducing antioxidant power
FTIR	Fourier transform infrared
FeSO <sub>4</sub> .7H <sub>2</sub> O	Hydrated iron sulphate
g	Gram
GC	Gas chromatography
GHI	Good honey index

HCA	Hierarchical cluster analysis
HCl	Hydrochloride
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HNO <sub>3</sub>	Nitric acid
HMF	Hydroxymethylfurfural
HPLC	High performance liquid chromatography
ICP-MS	Inductive coupled plasma mass spectrometry
KMO	Kaiser-Meyer-Olkin
Mg	Magnesium
mg	Milligram
mL	Milliliter
mm	Millimeter
Mn	Manganese
mM	Millimolar
MHA	Muller Hilton agar
MGO	Methylglyoxal
ms	Mass spectrometry
MS	Malaysian Standard
NaNO <sub>3</sub>	Sodium nitrate
NH <sub>2</sub>	Amine
NH <sub>3</sub>	Ammonia
Ni	Nickel
nm	Nanometer
NMR	Nuclear magnetic resonance

p	p-value
pH	pH
ppm	Part per million
RID	Reflective index detector
SCIRA	Stable carbon isotope ratio analysis
SPSS	Statistical Package for the Social Sciences
Sr	Serine
TPC	Total phenolic compound
TPTZ	2,4,6-Tri(2-pyridyl)-s-triazine
UMF	Unit manuka factor
UV	Ultraviolet
v/v	Volume over volume
w/v	Weight over volume
Zn	Zinc

## CHAPTER 1

### INTRODUCTION

Honeybees produce honey as a complex product which is one of humankind's oldest food. According to Codex Alimentarius Standard, honey is defined as the natural sweet element produced by honey bees from the nectar of flowers plant or secretion of parts of plants or excretions of the plant by sucker insects depend on the botanical origin (Standard and Honey, 2001). Honey is a viscous liquid with light brown and aroma composed sugar typically monosaccharides, and disaccharides compound also have trisaccharides and polysaccharides. Other minors compound including organics compound, amino acids, protein, vitamins, and enzymes.

Honey can be found regularly within in Malaysia country either it locally or imported from outside country. Common species of honeybee in Malaysia are such as *Apis mellifera*, *Apis cerana*, *Apis dorsata*, *Apis florea*, and *Apis koschevnikovi* (Moniruzzaman et al., 2013; Kek et al., 2016). Besides *Apis* sp., stingless bee species known as Meliponine can also be found in Malaysia. Stingless bee is getting more popular among beekeepers in Malaysia. Stingless bee has unique characters that determine their species. Honey produced by a stingless bee varies depending on their botanical origin, geographical origin, weather, and nectar source. Wei et al. (2018) reported that *Heterotrigona itama* and *Geniotrigona Thoracica* are the most species stingless bee that kept by beekeepers and 90% of honey sold in the market is from this species. Stingless bee honey price is \$100/kg compared to the price of *Apis* sp., which is (\$20-40/kg). Because of demanding stingless bee honey and economic competition, authenticity of the honey has been jeopardised. In order to check the purity of the honey, numerous analytical techniques have been applied to detect and quantify the adulterants that are present in the honey.

In year 2016, a minister from Ministry of Agriculture stated that Malaysia needs a standard for stingless bee honey to avoid fraud honey in the market. Thus in year 2017 Malaysia has released a standard called Malaysian Standard for stingless bee (MS 2683:2017). Continuity from this, requirement for stingless bee honey are equivalent and recognised to the people.

The variety of the characteristic and ranges of compound in stingless bee honey is other issue to identify which honey is a good quality honey. The quality are not standardised like Manuka honey that standardised by unit Manuka factor. Thus, honey is sold in the market have different quality even is comes same place. There is no grading system was introduced to control the grading of the honey. Effect from this, public are being deceived. In addition,

with the exist of the grading system, the fraudulent on the honey can be demolished.

Therefore objectives of this study are:

1. To characterize a comprehensive profiling of stingless bee honey.
2. To develop a Good Honey Index (GHI) for the stingless bee honey.



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Muhammad Fahdli Bin Abdul Rahman, raised in Merlimau, Melaka. He was born on 11<sup>th</sup> February 1992 at Malacca General Hospital. He is the youngest from five siblings. He had 3 brothers and 1 sister. He graduated his Bachelor of Science degree in Biochemistry from Universiti Putra Malaysia in 2016. He continued his Master Science in Biochemistry at the same university in 2016. He completed his MSc. in 2 years with one patented project for his master and was been awarded the EREKA 2018 Bronze medal for invention Good Honey Index.

Further, he is an active member of several Non-Government organisation such as Melaka Association Entrepreneur Meliponinie (MAEM). He is happily married to Fathin Nadia Norazif whom he met during his Matriculation studies.





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