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

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By

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

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Chair: Amir Syahir Amir Hamzah, PhD
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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 ≤ 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.

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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 pengambilam 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 ≤ 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 melepasi 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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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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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_2O	Deionized water
DPPH	2,2-diphenyl-1-picrylhydrazyl
FA	Factor analysis
Fe	Iron
FRAP	Ferric reducing antioxidant power
FTIR	Fourier transform infrared
$FeSO_4.7H_2O$	Hydrated iron sulphate
g	Gram
GC	Gas chromatography
GHI	Good honey index

HCA	Hierarchical cluster analysis
HCl	Hydrochloride
H_2O_2	Hydrogen peroxide
HNO_3	Nitric acid
HMF	Hydroxymethylfurfural
HPLC	High performance liquid chromatography
ICP-MS	Inductive coupled plasma mass spectometry
KMO	Kaiser-Meyer-Olkin
Mg	Magnesium
mg	Milligram
mL	Mililiter
mm	Milimeter
Mn	Manganese
mM	Milimolar
MHA	Muller Hilton agar
MGO	Methylglyoxal
ms	Mass spectrometry
MS	Malaysian Standard
$NaNO_3$	Sodium nitrate
NH_2	Amine
NH_3	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 decieved. 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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