THE PHYSICOCHEMICAL CHARACTERIZATION AND THE EFFECT OF STORAGE CONDITIONS ON QUALITY OF JOHOR STINGLESS BEE HONEY

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ABSTRACT

Stingless bee is the most commonly reared here in Malaysia for its beneficial and profitable honey. However, the information on physical and chemical characteristics of the stingless bee honey is still limited, because of limited honey production due to the physical characteristic of the bee. Besides that, the need concerning the most effective storage conditions for stingless bee honey arises since storage of honey is important to maintain the nutritional benefits of the honey. Therefore, the objectives of this study aimed to determine the physicochemical characterization and to investigate the effect of storage conditions on Johor stingless bee honey quality. This study represents the characteristics of 27 stingless bee honey samples, obtained from 10 districts of Johor, Malaysia. This study was first to characterized Johor stingless bee honey quality by evaluating the physical parameters and chemical parameters of the stingless bee honey. After that, the effect of the temperature (4°C, 24°C and 35°C) and duration (30 to 90 days) of storage on the selected Johor stingless bee honey sample were examined using three parameters, moisture content, acidity and hydroxymethylfurfural (HMF) content. Physicochemical parameters were assessed in triplicates and the data were analysed statistically. The results showed a correlation between moisture content with total soluble solids and carbohydrate, color with total phenolic content, HMF content with pH, while 2-2-diphenyl-1-picrylhydrazyl radical scavenging activity was not correlated with both total phenolic and flavonoid content. Principal component analysis presented that the stingless bee honey distributions from the same districts were distinguishable. The experiment on the effect of storage conditions on Johor stingless bee honey showed a strong correlation between storage conditions with moisture content, acidity, and HMF content which showed that the changes in the nutritional characteristics of the stingless bee honey was influenced by the storage. All parameters showed the smallest increased in 30 days at 4°C respectively, hence were the most desirable conditions. The findings from this study were expected to contribute useful information for the physicochemical standardization of stingless bee honey in Malaysia especially in Johor and help the Malaysian stingless bee honey industry to control safety, quality, and efficacy of honey.

ABSTRAK

Lebah kelulut adalah jenis lebah yang paling banyak diternak di Malaysia kerana manfaat dan keuntungan madu tersebut. Bagaimanapun, maklumat mengenai ciri-ciri fizikal dan kimia madu lebah kelulut masih terhad, oleh sebab pengeluaran madu yang sedikit disebabkan oleh ciri fizikal lebah tersebut. Selain itu, keperluan mengenai keadaan penyimpanan yang paling berkesan untuk madu kelulut timbul kerana penyimpanan madu adalah penting untuk mengekalkan faedah pemakanan madu. Oleh itu, objektif kajian ini adalah untuk menentukan pencirian fizikokimia dan untuk mengetahui kesan keadaan penyimpanan terhadap kualiti madu lebah kelulut Johor. Kajian ini menunjukkan ciri-ciri 27 sampel madu lebah kelulut, yang diperoleh dari 10 daerah di Johor, Malaysia. Kajian ini adalah untuk mengenal pasti kualiti madu lebah kelulut Johor dengan menilai parameter fizikal dan parameter kimia madu lebah kelulut tersebut. Selepas itu, kesan suhu (4 °C, 24 °C dan 35 °C) dan jangka masa (30 hingga 90 hari) penyimpanan pada sampel madu lebah kelulut Johor yang dipilih diperiksa menggunakan tiga parameter, kandungan kelembapan, keasidan dan kandungan hidroksimetilfurfural (HMF). Parameter fizikokimia dinilai dalam tiga kali bacaan dan data dianalisis secara statistik. Keputusan menunjukkan hubung kait antara kandungan kelembapan dengan jumlah pepejal larut dan karbohidrat, warna dengan jumlah kandungan fenolik, kandungan HMF dengan pH, manakala aktiviti pemerangkapan radikal 2-2-bifenil-1-pikrilhidrazil tidak dikaitkan dengan kandungan fenolik dan flavonoid. Analisis komponen utama menunjukkan bahawa pengagihan madu lebah kelulut dari daerah yang sama dapat dibezakan. Kajian mengenai kesan keadaan penyimpanan sampel madu lebah kelulut Johor yang dipilih menunjukkan hubung kait yang kuat antara keadaan penyimpanan dengan kandungan lembapan, keasidan dan kandungan HMF yang menunjukkan bahawa perubahan ciri pemakanan madu lebah kelulut dipengaruhi oleh penyimpanan. Semua parameter masing-masing menunjukkan peningkatan paling sedikit dalam 30 hari pada 4°C, oleh itu ini adalah keadaan yang paling diingini. Dapatan kajian ini dijangka menyumbangkan maklumat yang berguna bagi piawaian fizikokimia madu lebah kelulut di Malaysia terutamanya di Johor dan membantu industri madu lebah kelulut Malaysia untuk mengawal keselamatan, kualiti dan keberkesanan madu.

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LIST OF ABBREVIATIONS

2D	-	2-Dimensional
2FI	-	2 Factor Interaction
ABTS	-	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
ANOVA	-	Analysis of Variance
AOAC	-	Association of Official Agricultural Chemists
BBD	-	Box-Behnken Design
CCD	-	Central Composite Design
ChaGo-I	-	Lung undifferentiated cancer cell line
CHO	-	Carbohydrate
CTGase	-	Cyclodextrin glycosyltransferase
CODEX	-	Particle Swarm Optimization
DF	-	Degree of freedom
DN	-	Diastase Number
DOE	-	Design of Experiment
DPPH	-	2,2-diphenyl-1-picrylhydrazyl
E.C	-	Electrical conductivity
EHC	-	European Honey Commission
FA	-	Free acidity
GAE	-	Gallic acid equivalent
HCl	-	Hydrochloric acid
HepG2	-	Human liver cancer cell line
HMF	-	Hydroxymethylfurfural
HPLC	-	High-Performance Liquid Chromatography
HSD	-	Honestly significance difference
IBM	-	International Business Machines
IC_{50}	-	Inhibition concentration minimized at 50%
IHC	-	International Honey Commission
JSBH	-	Johor Stingless Bee Honey
JMP	-	JUMP Statistical Software
KGaA	-	Kommanditgesellschaft auf Aktien (Merck Group)
LDL	-	Low Density Lipoproteins

MARDI	-	Malaysian Agricultural Research and Development Institute (MARDI)
Max	-	Maximum
MoA	-	Ministry of Agriculture and Agro-based Industry
Moi	-	Moisture
MSB	-	Mean value of the squares between groups
MSW	-	Mean value of the squares between groups
NaOH	-	Sodium hydroxide
OFAT	-	One-Factor-at-a-Time
ORAC	-	Oxygen Radical Absorbance Capacity
p	-	Probability
PABA	-	Para-aminobenzoic acid
PCA	-	Principal Component Analysis
PCs	-	Principal components
Prot	-	Protein
RE	-	Rutin equivalent
RSA	-	Radical Scavenging Activity
SD	-	Standard deviation
sp.	-	Species
SPSS	-	Statistical Package for the Social Sciences
SSB	-	Sum of the squares between groups
SSW	-	Sum of the squares within groups
TEAC	-	Trolox Equivalent Antioxidant Capacity
TFC	-	Total flavonoid content
TPA	-	Tissue polypeptide antigen
TPC	-	Total phenolic content
TSS	-	Total soluble solid
UV	-	Ultraviolet
UV-Vis	-	Ultraviolet-visible spectroscopy
XLSTAT	-	Microsoft Excel statistical analysis add-in

LIST OF SYMBOLS

β_o	-	Constant term
H_o	-	Null hypothesis
H_A	-	Alternative hypothesis
ε	-	Residual
x_k	-	Variables
µ1	-	Response variable
g	-	gram
h	-	hour
M	-	Molarity
n	-	Number of sample
N	-	Normality
r	-	Correlation coefficient
R^2	-	r-squared
kg	-	Kilogram
mg	-	Milligram
mL	-	Millilitre
mm	-	Millimetre
mm Pfund	-	Millimetre Pfund
nm	-	Nanometre
mg/kg	-	Milligram per kilogram
mg/L	-	Milligram/litre
mg/mL	-	Milligram per millilitre
mEq/kg	-	Milliequilibrium per kilogram
mEq/100 g	-	Milliequilibrium per hundred gram
mS/cm	-	milliSiemen per centimetre
µS/cm	-	Micrometre per centimetre
w/w	-	Weight per weight
w/v	-	Weight per volume
g/mL	-	Gram per millilitre
g/L	-	Gram per litre
g/100 g	-	Gram per hundred gram

rpm	-	Revolutions per minute
µm	-	Micrometre
α	-	Level of significance
%	-	Percentage
°Brix	-	Degree Brix
°C	-	Degree Celsius
®	-	Registered trademark symbol

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Food quality has been an important aspect to maintain the product's value especially to be used by the consumers. This could be meant by controlling the occurrence of the food from being spoiled due to the contamination by various microorganisms and filth, and also to retain the food quality by looking into consideration on the attribution of some factors such as color, taste, flavour, texture and processing method. Good quality of food usually follows the recommended standard by various organizations and they had listed various types of food according to the properties from handling to the storage of the food.

Honey, a gold-like liquid, has a great contribution in many fields of industries mainly food industries and medicine field. It can be used as a food sweetener, avoiding the sugar, heal wounds, act as an antibacterial agent and other advantages. The emergence of stingless bee honey was long before the great discovery of honeybee honey. However, the popularity of honeybee honey conceals the greatness of stingless bee honey. Honey utilization has been focusing on common honeybee honey from *Apis mellifera sp*. as the introduction of honey was first to come from these types of species. Only recently that the stingless bee honey emergence has come to the surface as one of the types of honey. In Malaysia, there are more than 30 species of stingless bee honey found and the beekeepers of stingless bee honey found in Malaysia to date were about 700 stingless beekeepers (Ismail and Ismail, 2018). Johor, as one of Malaysia's districts, also followed the call from the Ministry of Agriculture and Agri-Industry (MOA) to establish the National Industrial Development Plan for Stingless Bee Honey in the year of 2020-2030 in an attempt to advance and improve the country's international export industry.

Stingless bee honey is not very much different from *Apis mellifera* honey. because the bees produced honey from the nectar that was collected from floral sources and exudates of plant-sucking insects. The difference between these two types of honey is that only honey from *Apis mellifera* spp. was included in the standard (Alimentarius, 2001). Insufficient knowledge on stingless bee honey and at the same time the extensive study on the *Apis mellifera* honey lead to the This could be insufficient knowledge of the stingless bee honey. Hence, many studies had been done to determine and identify the characteristics of this honey based on the physicochemical properties of the honey. However, the researchers only identify the characteristics of the stingless bee honey from different types of species from different countries and no specific legislation had been produced yet. Therefore, the stingless bee honey quality is only based on how the honey will be handled from the caring of the bees until the storage of the honey. Fortunately, in the year of 2017, Malaysia had released the standard specifically for stingless bee honey which is the Malaysia Standard, MS 2683: 2017: Kelulut (Stingless bee) Honey-Specification (Standards Malaysia, 2017).

Storage of honey is an important aspect of maintaining the quality of the honey. Most of the time, beekeepers, suppliers, and consumers store their honey in a close tight container (glass or plastic) to prolong the shelf-life of the honey. A close tight container is functions to prevent the absorption of honey to the moisture outside of the bottle because honey is a good absorbent. Besides, if the water content is higher in the honey, the possibility for the fermentation by yeast is high. This is due to the properties of the yeast that favour the moisture environment. Therefore, the importance of monitoring the moisture content during storage is essential for maintaining the nutritional values in the stingless bee honey.

However, due to high moisture content, most of the beekeepers will apply heat to remove the water content. Heat exposure on the honey could cause the production of a toxic compound named hydroxymethylfurfural (HMF) compound. HMF compound is not only present in honey but could be formed in most of the foods. However, there are no studies proving the toxicity effects of the HMF compound in humans. The HMF compound is produced due to the breakdown of fructose in the

presence of the acid. This could be predicted that the fructose level will be lessened and there will be an increased in acidity level if the HMF content in honey is high. Hence, the storage time with the addition of the exposure to high temperature could be the cause of the deterioration of the honey which will then lower the honey quality.

The establishment of the quality standards specifically for Malaysian stingless bee honey has been developed recently. Determination of characteristics of Johor stingless bee honey is important as additional future data for the standard regulation. In addition to that, the honey also needs to be stored very well to ease the process during transportation of the honey until the storage of the honey. The maintenance of the stingless bee honey in terms of its storage condition can be used to help for a better quality of Johor stingless bee honey.

1.2 Problem Statement

The rearing of stingless bees for the purpose of obtaining their honey has been increased in all states in Malaysia including Johor. Most of the study on Malaysian stingless bee honey was evaluating on the characterization of honey at the most renounce rearing locations or comparison of the characterization of stingless bee honey based on different types of the species. The information on this type of honey has been profoundly studied, but the information of Johor stingless bee honey characteristics is still unknown. The establishment of nutritional characterization is very important

In addition, storage conditions of stingless bee honey were important to maintain the quality of honey for better consumption. A concern appears for the stingless bee honey concerning the problem faced by the honey during the storage of the honey. Storage condition like long-term storage with thermal exposure reduced the nutritional quality of stingless bee honey. However, to maintain the appropriate temperature and duration during storage is difficult due to our climate condition (high humidity level and temperatures averaging between 28°C - 30°C, thus could lower the quality of the honey

Most of the time, consumers would like to obtain the honey freshly. However, honey could face certain negative conditions. These conditions are fermentation, honey more acidic, and the probability of formation of the toxic compound like hydroxymethylfurfural (HMF) compound that may cause a carcinogenic effect to the human. In Malaysia, there has been little research for the study of storage conditions of stingless bee honey focusing on its freshness and quality. The factors involved are moisture content, acidity, and hydroxymethylfurfural (HMF) content. In some study, the storage conditions on Malaysian stingless bee honey was studied on the various factors. However, the study on storage conditions and its relationship with the quality of Johor stingless bee honey is still remained unknown. This information is important to help the stingless bee honey industry specifically in Johor for beekeepers, manufacturing and consumer.

Moreover, there is a very limited study on the effect of storage conditions on Malaysian stingless bee honey using computer-generated statistical design methodology. This method was effective compared to the traditional method such as one-factor-at-a-time (OFAT) design by offering shorter time (low number of the experimental run), cost-effective and precise and accurate data analysis. The present study attempts to determine the best storage conditions of stingless bee honey by using a computer-generated design method and provide guidance for the development of the Malaysian stingless bee honey industry.

1.3 Objective of the Study

The objective of this study was:

i. To determine the physicochemical characterization of Johor stingless bee honey.

ii. To evaluate the effect of temperature and duration of storage and its relationship with quality of stingless bee honey.

1.4 Scope of the Study

i. Determination of the physicocharacteristics of 27 stingless bee honey collected from 26 places in 10 districts of Johor by physical characterization (pH, moisture, ash, electrical conductivity, and acidity) and chemical characterization (total phenolic content, total flavonoid content, antioxidant activity, and hydroxymethylfurfural (HMF) content).

ii. Evaluation of the correlation between physical and chemical characteristics of Johor stingless bee honey by statistical analysis (Pearson's correlation coefficient) and Principal Component analysis to get the best stingless bee honey sample

iii. Determination of the effect of temperature (4°C, 24°C, and 35°C) and duration (30 to 90 days) of storage on Johor stingless bee honey quality (moisture content, acidity and hydroxymethylfurfural (HMF) content) by using Factorial design (Design-Expert Version 6.0.6 software).

iv Evaluation of the correlation between storage conditions (duration and temperature) of selected stingless bee honey sample by Pearson's correlation coefficient.

1.5 Significance of the Study

Stingless bee honey has long been introduced to the consumer due to its beneficial purposes such as therapeutic effects. The quality of this type of honey is important since this honey will be used by consumers in daily life. Besides, the advantages of consuming stingless bee honey were studied in most researches that can lead to a healthy lifestyle of the consumers. An increase in awareness and demands for more stingless bee honey can further improve the production and maintenance of the stingless bee honey.

The producers and marketers of Johor stingless bee honey may, therefore, act in response to the demands from the consumers more quickly by following the guideline of production and storage of the honey. The improvement of the quality of Johor stingless bee honey to gain consumer confidence will, therefore, follow the policy which stated that stingless bee honey is known as a superfood that was announced by the Ministry of Agriculture and Agro-based Industry (MoA) (MARDI, 2016). Meanwhile, the benefits of stingless bee honey also can help most researchers to develop stingless honey bee-based products that can benefits the consumers, increase the stingless bee honey industry to the highest and improve the economic status of Malaysia stingless bee honey towards better future.

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1. Ya'akob, H**.**, Norhisham, N.F., Mohamed, M., Sadek, N. & Endrini, S. (2019) Evaluation of Physicochemical Properties of Trigona sp. Stingless Bee Honey from Various Districts of Johor. *Jurnal Kejuruteraan*, *2*(1), 59-67. https://doi.org/10.17576/jkukm-2019-si2(1)-08. **(Indexed by WOS-ESCI)**