THERMAL TREATMENT EFFECT ON THE BIOCHEMICAL COMPOSITION OF HONEY SAMPLES

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To my loving parents,
Adnan Mohd Zin and Zurida Kamaruddin,
my sisters and my brother

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ABSTRACT

Thermal treatment of honey is commonly carried out to prevent honey from fermentation, crystallization and to facilitate bottling. However, this treatment is known to change the biochemical composition of honey and its quality. In the present study, three honey samples, namely the Tualang, Gelam and Acacia honey were pasteurized at 63 °C for 30 minutes and heat treated at 90 °C for 60 minutes. The study showed that heat treatment at 90 °C up to 30 minutes successfully reduced the moisture content of honey samples to the range of 17.0-19.8 %. The moisture content of less than 20 % is required to reduce the rate of fermentation and crystallization. The biochemical composition of fresh and thermal treated honey samples (90 °C for 30 minutes) such as macro-nutrients (sugars, crude protein, diastase, invertase, glucose oxidase, amino acids and crude fat) and micro-nutrients (minerals and water-soluble vitamins) were analysed and compared statistically. The result of the Principal Component Analysis (PCA) highlighted that diastase, panthotenic acid and crude fat were the most affected parameters after thermal treatment with the first principal component covered for 86.4 % of the total variance. The degradation of diastase and invertase due to the thermal proteolytic digestion contributed to the increment of most amino acids, particularly cystine, histidine, tyrosine and valine. In general, Tualang, Gelam and Acacia honey samples responded differently towards thermal treatment. This was because of the difference in their initial biochemical composition.

ABSTRAK

Rawatan haba sering dijalankan pada madu untuk mencegah proses fermentasi dan penghabluran, selain daripada membantu dalam proses pembotolan. Walaubagaimanapun, rawatan haba boleh mengubah komposisi biokimia dalam madu dan kualiti madu tersebut. Dalam kajian ini, tiga sampel madu iaitu Tualang, Gelam dan Akasia telah dipasteurkan pada suhu 63 °C selama 30 minit dan telah diberi rawatan haba pada suhu 90 °C selama 60 minit. Kajian ini menunjukkan bahawa rawatan haba pada 90 °C selama 30 minit telah berjaya mengurangkan kandungan air ke tahap 17.0-19.8 %. Kandungan air kurang daripada 20 % diperlukan untuk mengurangkan kadar fermentasi dan penghabluran. Analisis komposisi biokimia yang diklasifikasikan kepada makro-nutrien (gula, protin kasar, enzim diastase, invertase dan glukosa oksidase, asid amino dan lemak kasar) dan mikro-nutrien (mineral dan vitamin larut air) telah dijalankan terhadap sampel madu sebelum dan selepas rawatan haba (90 °C, 30 minit). Semua data telah dibandingkan secara statistik. Analisis Komponen Utama (PCA) mendapati bahawa enzim diastase, asid pantotenik dan kandungan lemak kasar mengalami penyusutan ketara selepas rawatan haba dengan komponen utama pertama merangkumi 86.4 % daripada varian keseluruhan. Penyusutan enzim diastase dan invertase melalui proteolisis haba telah menyumbang kepada kenaikan kepekatan kebanyakan asid amino, terutamanya sistina, histidina, tirosina dan valina. Secara keseluruhan, sampel madu Tualang, Gelam dan Akasia telah menyumbang kepada respon yang berbeza terhadap rawatan haba yang dijalankan kerana perbezaan komposisi biokimia yang asal dalam sampel madu tersebut.

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LIST OF ABBREVIATIONS

°C	-	Degree celcius
%	-	Percentage
n	-	Number of samples
min	-	Minute
α	-	Alpha
β	-	Beta
DN	-	Diastase number
U/kg	-	Enzyme unit per kilogram
n.d	-	Not detected
M	-	Molarity
H_2O_2	-	Hydrogen peroxide
g/100g	-	Gram per 100 gram
ppm	-	Part per million
g	-	Gram
mg/kg	-	Miligram per kilogram
mg	-	Miligram
$CuSO_4$	-	Copper sulphate
K_2SO_4	-	Potassium sulphate
ml	-	mililitre
H_2SO_4	-	Sulphuric acid
H_2O	-	Water
NaOH	-	Sodium hydroxide
HCl	-	Hydrochloric acid
N	-	Normality

T	-	Sample titration volume
B	-	Blank titration volume
F	-	Crude protein factor
nm	-	Nanometer
>	-	More than
<	-	Less than
ΔA	-	Absorbance
™	-	Trademark
µM	-	Micromolar
µl	-	Microlitre
OD	-	Optical density
U/L	-	Enzyme unit per litre
t	-	Time
U/ml	-	Enzyme unit per mililitre
KH_2PO_4	-	Monopotassium phosphate
K_2HPO_4	-	Dipotassium phosphate
mg/ml	-	Miligram per mililitre
BSA	-	Bovine serum albumin
mg/l	-	Miligram per litre
v/v	-	Volume per volume
rpm	-	Revolution per minute
µm	-	Micrometer
mm	-	Milimeter
ml/min	-	Mililitre per minute
MeOH	-	Methanol
ACN	-	Acetonitrile
SD	-	Standard deviation
R^2	-	Determination coefficient
$mM^{-1}cm^{-1}$	-	Mili per molar per centimeter
μM^{-1}	-	Micro per molar

CHAPTER 1

INTRODUCTION

1.1 Research Background

Honey is a natural sweetener that produced by honeybees and it offers many beneficial effects to human beings both as food and medicine. Sugar and water are the major components in honey (Cavia *et al*., 2002), besides the other minor substances such as proteins, amino acids, enzymes, lipids, vitamins and minerals (Hebbar *et al*., 2003). Previous studies revealed the therapeutic application of honey in wound healing, gastric ulcers, diabetes and as a cancer cure (Tsiapara *et al*., 2009). The therapeutic potential of honey is recognized since the ancient time.

However, the quality of honey deteriorates gradually due to honey fermentation (Ghazali *et al*., 1994) and honey crystallization which is usually happening to the unprocessed honey from tropical countries. This is because rainy and wet weather in Malaysia always resulted to incomplete honey evaporation (Kretavicius *et al*., 2010). Besides, honey contains sugar-tolerant yeasts that also contributing to honey fermentation (Subramanian *et al*., 2007).

In order to prolong the shelf life of honey, thermal treatment is commonly applied to prevent honey from fermentation and crystallization, as well as to eliminate microorganisms by reducing the moisture content of honey. However, thermal treatment might change the biochemical composition in honey (Khan *et al*., 2007). The scientific data regarding the biochemical composition of honey, particularly on Malaysian honey after thermal treatment is very limited. Therefore, this study is important to determine the honey quality based on the biochemical components.

1.2 Problem Statement

Previous studies on the effect of thermal treatment to the biochemical composition of foreign honey samples have been well-developed. Many standards and regulations have been established for foreign honey to monitor honey quality such as the Codex European Regional Standard (White, 1992), European Directive, Codex Alimentarius Standard and International Honey Commission. However, there are limited studies on the biochemical composition of Malaysian honey in relation with thermal treatment. In addition, the major biochemical component affected by thermal treatment is also not widely investigated.

The limited studies using local honey samples have caused the difficulties in monitoring local honey quality. Thermal treatment has been applied at different temperatures and duration to reduce the moisture content to less than 20% (Subramanian *et al*., 2007). Most of studies that conducted for foreign honey samples are not suitable as reference for local honey. This is because of large variance in biochemical composition of honey. Malaysian honey usually contains high moisture content compared to foreign honey due to high average rainfall

throughout the year. Therefore, this study is required to establish the biochemical profile of local honey samples after thermal treatment.

1.3 Importance of Study

Thermal treatment is commonly applied process to honey in Malaysia in order to prevent honey fermentation and honey crystallization, as well as to facilitate bottling. Although thermal treatment is important, it still might affect the biochemical composition of honey, and simultaneously the honey quality. However, the scientific data on the biochemical composition of local honey by thermal treatment is very limited. Therefore, this study is important to be carried out since it is beneficial as reference, especially for local bee keepers and honey consumers. From the findings, the biochemical component that mostly affected by thermal treatment can be identified.

Besides, the findings can be a reference to improve the local apicultural industry. The relationship between honey quality and thermal treatment is crucial for honey processing and production. In fact, this study is in line with the aspiration of Malaysian government to promote local industry including local apicultural industry.

1.4 Objective of Study

The objective of this study was to investigate the effect of thermal treatment on the biochemical composition of local honey samples such as Tualang, Gelam and Acacia honey.

1.4.1 Scopes of Study

The scopes of the study can be divided into two categories;

i. To determine the concentration of macro- and micro-nutrients in local honey samples before and after thermal treatment.
ii. To investigate the most affected biochemical parameters due to thermal treatment by using multivariate data analysis approach.

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