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EFFECTS OF THERMAL PROCESSING AND THERMOSONICATION ON QUALITY OF HONEY FROM STINGLESS BEES

CHONG KAR YEEN

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By

CHONG KAR YEEN

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EFFECTS OF THERMAL PROCESSING AND THERMOSONICATION ON QUALITY OF HONEY FROM STINGLESS BEES

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April 2017

Chairperson: Professor Ir. Chin Nyuk Ling, PhD
Faculty: Engineering

One of the distinctive characteristics of stingless bee honey is its higher moisture content than honeybee honey. Honey being a supersaturated sugar solution tends to crystallize and ferment easily. The objective of this research was to study the effects of thermal processing and thermosonication on the quality of a stingless bee honey in Malaysia, the *Kelulut*. A two-factor-five-level design was adopted and the factors were processing temperature ranging from 45 to 90 °C and processing time ranging from 30 to 120 minutes for both methods. Physicochemical properties including water activity, moisture content, colour intensity, viscosity, hydroxymethylfurfural (HMF) content, total phenolic content (TPC), and radical scavenging activity (RSA) were determined. Thermosonicated honey had its water activity and moisture content reduced by 7.9% and 16.6%, respectively compared to 3.5% and 6.9% by thermal processing. Thermosonicated honey had its colour intensity increased by 68.2 %, viscosity increased by 275.0%, TPC increased by 58.1%, and RSA increased by 63.0% when compared to its raw form. The increase in HMF to 62.46 mg/kg using thermosonication was within the limits of international standards. The second objective of this study was to optimise thermal processing and thermosonication conditions using response surface methodology (RSM) based on minimum water activity, moisture content, and HMF content while maximizing colour intensity, viscosity, TPC, and RSA. The optimum conditions for thermal processing were at 90 °C for 108 minutes while for thermosonication it was at 90 °C for 111 minutes. To examine potential anti-inflammatory effects of the honey samples, the ability of the optimized *Kelulut* honey to inhibit nitric oxide production in lipopolysaccharide (LPS)-stimulated murine macrophages, RAW 264.7 cells, was evaluated. Results showed that *Kelulut* honey was able to both inhibit and stimulate nitric oxide using honey concentrations of 10, 20, and 50 µg/mL. Additionally, *Kelulut* honey promotes cell growth of RAW 264.7 cells. Thermosonication was revealed to be an effective honey processing alternative to current practices and the use of *Kelulut* honey as a functional food is proposed.

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

KESAN PEMPROSESAN TERMA DAN TERMOSONIKASI KE ATAS KUALITI MADU KELULUT

Oleh

CHONG KAR YEEN

April 2017

Pengerusi: Profesor Ir. Chin Nyuk Ling, PhD
Fakulti: Kejuruteraan

Salah satu ciri-ciri unik madu Kelulut adalah bahawa ianya mempunyai kandungan air yang lebih tinggi daripada madu lebah. Madu adalah sejenis larutan gula tepu yang mudah berubah menjadi hablur dan menjalani proses fermentasi. Objektif penyelidikan ini adalah untuk mengkaji kesan pemprosesan terma dan termosonikasi ke atas kualiti madu Kelulut. Eksperimen yang direka untuk kajian ini menggunakan dua faktor dan lima paras. Faktor-faktornya adalah suhu pemprosesan dari 45 ke 90 °C dan jangkamasa pemprosesan dari 30 ke 120 minit untuk kedua-dua jenis pemprosesan yang dinyatakan. Sifat-sifat fizikokimia seperti aktiviti air, kandungan air, intensiti warna, kelikatan, kandungan hydroxymethylfurfural (HMF), kandungan jumlah fenolik, dan aktiviti pemerangkapan radikal dikaji. Termosonikasi mampu mengurangkan aktiviti air dan kandungan air masing-masing sebanyak 7.9% dan 16.6% berbanding dengan 3.5% and 6.9% apabila pemprosesan terma digunakan. Termosonikasi juga meningkatkan intensiti warna sebanyak 68.2%, meningkatkan kelikatan sebanyak 275.0%, meningkatkan kandungan jumlah fenolik sebanyak 58.1%, dan meningkatkan aktiviti pemerangkapan radikal sebanyak 63.0% apabila dibandingkan dengan madu Kelulut yang tidak diproses. Peningkatan kandungan HMF kepada 62.46 mg/kg apabila termosonikasi digunakan masih berada di bawah had maksimum yang ditetapkan oleh piawaian antarabangsa. Objektif kedua kajian ini adalah untuk mengoptimakan suhu dan jangkamasa pemprosesan terma dan termosonikasi berdasarkan paras minimum aktiviti air, kandungan air, dan kandungan HMF manakala intensiti warna, kelikatan, kandungan jumlah fenolik, dan aktiviti pemerangkapan radikal dimaksimakan. Keadaan optima untuk pemprosesan terma adalah dengan menggunakan suhu 90 °C selama 108 minit manakala untuk termosonikasi, suhu 90 °C selama 111 minit disyorkan. Untuk menguji potensi sifat anti-keradangan dalam sampel madu Kelulut, keupayaan madu Kelulut yang telah dioptimakan untuk menyekat pengeluaran oksida nitrik dalam sel makrofaj murin, sel RAW 264.7 telah dikaji. Hasil kajian menunjukkan bahawa madu Kelulut mempunyai keupayaan untuk menyekat dan merangsang pengeluaran oksida nitrik apabila kepekatan madu sebanyak 10, 20, dan 50 μg/mL digunakan. Tambahan pula, madu

Kelulut menggalakkan pertumbuhan sel RAW 264.7. Termosonikasi terbukti sebagai satu kaedah pemprosesan madu alternatif kepada pemprosesan semasa manakala penggunaan madu Kelulut sebagai makanan fungsional disarankan.

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REPLACE!

I certify that a Thesis Examination Committee has met on 17th April 2017 to conduct the final examination of Chong Kar Yeen on her thesis entitled "Effects of Thermal Processing and Thermosonication on Quality of Honey from Stingless Bees" 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 Degree of Master of Science.

Members of the Thesis Examination Committee were as follows:

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Universiti Putra Malaysia
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Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Internal Examiner)

Ida Idayu Muhammad, PhD
Professor
Department of Bioprocess & Polymer Engineering
Faculty of Chemical & Energy Engineering
Universiti Teknologi Malaysia
(External Examiner)

Nor Aini Ab. Shukor, PhD
Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

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:

Ir. Chin Nyuk Ling, PhD
Professor
Faculty of Engineering
Universiti Putra Malaysia
(Chairperson)

Yus Aniza binti Yusof, PhD
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)

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Associate Professor
Faculty of Medicine and Health Sciences
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Signature: ______________________
Name of
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Committee: ______________________

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
DMSO	Dimethyl sulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
HMF	Hydroxymethylfurfural
LPS	Lipopolysaccharide
MRP	Maillard reaction product
MTT	Methylthiazolyldiphenyl-tetrazolium bromide
NO	Nitric oxide
PBS	Phosphate buffered saline
RSA	Radical scavenging activity
RSM	Response surface methodology
TPC	Total phenolic content

CHAPTER 1

INTRODUCTION

1.1 Significance of Honey

Honey is used for many purposes. It can be consumed as it is, be used as spread, or directly added to sweeten drinks. In bakery products such as *baklava*, a Turkish flaky pastry, gingerbreads, and fruit cakes, honey is used as it improves moisture retention and thus increases shelf life (Tong *et al.*, 2010). Apart from giving cakes a richer flavour, honey eliminates dryness and crumbliness in them. Honey is also used in confectionery such as caramel and nougat. One of the pull factors is the nutritional benefits of honey, as it is often used for medicinal purposes. Some of the medicinal uses of honey include treatment of digestive disorders, respiratory infections, and promotion of wound healing (Vit, Medina, & Eunice Enríquez, 2004). Home remedies also use honey to soothe sore throats and coughs. Honey is also integrated into cosmetics and skincare products as it has antioxidant and hygroscopic properties, thus providing a soothing effect on the skin.

In the wild, honeybees usually nest in hollow trees or rock crevices rather high above the ground. Over the years, honey hunting shifted to beekeeping in manmade hives where they can be conveniently accessible and safe from predators. This transition was important to increase honey production as its demand increased. From immovable combs, honey production advanced to movable-frame hive, and subsequently embossed beeswax foundation was invented to fit into the frames (Crane, 1980). A centrifugal extractor would spin the honey out and the frames are ready to be reused. This method is still presently used by small-scaled and artisanal honey producers.

Stingless bees which are much smaller in size, were prized for its cerumen and honey in native civilizations. The evolution from honey hunting to beekeeping is similar for stingless bees. From wild bees in hollow trees, beekeeping evolved to usage of a hollow log. The hollow log has a central flight entrance and closure and it was cut in such a way that it can be opened and resealed by the owner (Jones, 2013). At present, log hives are used along with boards.

Figure 1.1: Honey pots made by stingless bees

The tropical climate in Malaysia enables various plants to flourish which in turn becomes a suitable environment for foraging bees. Honey can be broadly categorized by its botanical origin and type of bees. Common honey found in Malaysia are the Acacia, Pineapple, *Tualang*, Borneo, *Gelam*, and *Kelulut* honey (Chua, Abdul-Rahaman, Sarmidi, & Aziz, 2012; Kek, Chin, Tan, Yusof, & Chua, 2016; Moniruzzaman, Khalil, Sulaiman, & Gan, 2013). The *Kelulut* honey is sourced from *Trigona* spp. stingless bees. It is a multifloral honey and some of the unique characteristics of stingless bee honey or 'pot honey' are its higher moisture content, higher electrical conductivity, higher acidity, and lower diastase activity (Chuttong, Chanbang, Sringarm, & Burgett, 2016).

1.2 Production of Stingless Bee Honey and Common Problems

Similar to other bees, stingless bees collect nectar and pollen from flowers and carry them to the nest where the larvae are fed. The food goes into cerumen pots. At the hive, the bees ripen or dehydrate the nectar droplets by spinning them inside their mouthparts until honey is formed. Honey harvest was done by piercing or squeezing the pots of honey and pollen. Modern technology then introduced the use of a suction device to improve product quality. However, low productivity of stingless bee colonies is one of the factors deterring beekeepers and consumers (de Oliveira Alves, 2013).

Both stingless bee honey and honeybee honey have natural tendency to undergo crystallization and fermentation. Honey is a highly-saturated sugar solution and crystallization will occur spontaneously. When crystals are formed, two distinct phases which are crystallized phase at the bottom and liquid phase at the top can be seen. This undesirable appearance will deter consumers from purchasing the honey. Besides that, an increase in water activity becomes a favourable environment for naturally-present osmophilic yeasts in honey to multiply. As stingless bee honey itself has higher moisture content, fermentation degrades honey quality by altering its taste (Gleiter, Horn, & Isengard, 2006).

Current honey production practices by the beekeepers include refrigeration, dehydration, pasteurization, and maturation. Refrigeration involves keeping honey at approximately 4-8 °C just after harvesting and until consumption. Dehydration involves ventilating the honey in a dry room with a dehumidifier. Pasteurization is also performed at 72 °C for 15 seconds to eliminate pathogens. Another method is to develop maturity of the honey. Maturation is done by keeping honey inside closed bottles, and opened once a week to release gases produced by fermentation, and closed again. This process continues until no more gas is released. Then, the stabilized honey can be stored (Menezes, Vollet-Neto, Contrera, Venturieri, & Imperatriz-Fonseca, 2013).

Honey is well-known for its many benefits such as high antioxidants content, antimicrobial activity, wound-healing, and anti-inflammatory activities (Liu, Ye, Lin, Wang, & Peng, 2013). Further explanation can be found in Section 2.1.5. Hence, honeybee honey is often tested for its anti-inflammatory activities (Van den Berg et al., 2008). There are studies which used Malaysian honey such as Gelam honey to conduct *in vitro* and *in vivo* anti-inflammatory tests (Kassim, Achoui, Mansor, & Yusoff, 2010a; Kassim, Achoui, Mustafa, Mohd, & Yusoff, 2010b). However, there are insufficient studies on Kelulut honey and it is important to investigate the therapeutic properties to increase its consumption.

1.3 Research Objectives

The objectives of this research were:
i. to study the effects of temperature and time on the quality of *Kelulut* honey using thermal processing and thermosonication,
ii. to optimize thermal processing and thermosonication by using response surface methodology, and
iii. to evaluate the anti-inflammatory activity of selected treated honey by investigating inhibition of nitric oxide production in RAW 264.7 cells.

1.4 Thesis Scope and Organization

Although there are many by-products of honey production such as propolis, bee pollen, royal jelly, beebread, and cerumen, this study focused only on honey. The processing methods used were conventional thermal processing and thermosonication. Honey quality was evaluated by its physicochemical properties covered by this research which were water activity, moisture content, colour intensity, viscosity, hydroxymethylfurfural content, total phenolic content, and radical scavenging activity. One of the ways to screen anti-inflammatory activities is by studying the inhibition of nitric oxide production in lipopolysaccharide-stimulated cells. For this purpose, mouse leukemic macrophages, also known as RAW 264.7 cells, were used.

The thesis comprises of seven main chapters. Chapter One gives an overview of the research, a brief introduction of objectives, and the problems encountered which led to initiation of this research. Chapter Two reviews literature of honey composition, physicochemical properties of honey, anti-inflammatory activities of honey, detailed explanation on honey crystallization and fermentation, and effects of thermal processing and thermosonication on honey. Chapter Three focusses on the methodology including the experimental design and materials used for the entire research. Chapter Four presents the results and the effects of thermal processing and thermosonication on the quality of *Kelulut* honey. Chapter Five discusses optimization of honey quality. By using face-centred central composite design in the Minitab Statistical Software 16, the surface regression analysis, modelling response, surface plots, optimisation, and desirability of each response were obtained and discussed. Chapter Six concentrates on the investigation of anti-inflammatory properties of processed honey. It comprises of a cell viability assay and a nitric oxide assay. Chapter Seven summarizes the results and concludes the entire research.

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