HIGH-PRESSURE PROCESSING (HPP) OF KELULUT HONEY (SARAWAK): ENHANCEMENT ON THE QUALITY AND ALTERATION OF PREBIOTIC POTENTIAL ON *LACTOBACILLUS*

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Dedicated to my beloved parents,

For their unconditional love and support

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ABSTRACT

Kelulut honey is an elixir containing various beneficial compounds and has a potential of prebiotic. Conventional thermal treatments in honey industries caused more harm than good to thermolabile components in honey. Therefore, high-pressure processing (HPP) was chosen as it does not exert significant heat thus minimize any damaged done. HPP was carried out at two different pressures (200 and 600 MPa) with two different holding times respectively (5 and 10 minutes). Conventional thermal treatments (60 and 90 °C) were also carried out for comparison. HPP in general produces refined Kelulut honey. With exception to colour, HPP demonstrated good retention of total acidity, reducing sugar, total sugar and diastase activity. Astoundingly, HPP at 600 MPa/10 minutes managed to increase the antioxidant activity and total phenolic content (TPC) by 3.0% and 47.2% respectively. Furthermore, HPP-treated at 600 MPa/10 minutes was successfully retaining the prebiotic characteristic given the good retention of total carbohydrates. Intriguingly, restricted growths were observed in *Lactobacillus* strains grown with HPP-treated at 600 MPa/10 minutes as evident by longer (> 8 h) doubling time (T_d). Enhanced TPC was deduced as the inhibitor supported by the strong correlation between TPC and doubling time ($r > 0.8$). Nonetheless, HPP was able to produce refined quality of Kelulut honey with enhanced total phenolic content, outstanding antioxidant activities and good retention of prebiotic characteristics. In a nutshell, HPP has established itself as the better processing method for Kelulut honey than thermal processing and thus has an implication on postharvest enhancement and extraction of bioactive compound.

ABSTRAK

Madu Kelulut merupakan cecair semulajadi yang mengandungi banyak khasiat dan berpotensi sebagai sumber prebiotik. Rawatan haba secara konvensional yang selalu diguna pakai dalam pemprosesan madu mendatangkan lebih banyak kesan buruk terhadap komponen sensitif haba. Oleh itu Pemprosesan Tekanan Tinggi (HPP) dipilih sebagai rawatan alternatif berdasarkan sifatnya yang tidak mengenakan haba lantas mengurangkan kerosakan semasa pemprosesan. HPP dijalankan menggunakan dua tahap tekanan (200 and 600 MPa) dengan dua tempoh masa yang berbeza (5 dan 10 minit). Rawatan haba secara konvensional turut dijalankan bagi tujuan perbandingan. Hasil daripada dapatan kajian menunjukkan madu rawatan HPP lebih berkualiti berbanding madu rawatan haba. HPP menunjukan pengekalan sifat-sifat semulajadi madu seperti tahap keasidan, isi kandungan gula penurun, dan aktiviti enzim diastase. Menariknya, rawatan HPP ditahap 600 MPa/10 minit menunjukkan peningkatan aktiviti antioksidan dan kandungan fenolic sebanyak 3% dan 47.2% . Selain itu HPP ditahap 600 MPa/10 minit juga mengekalkan ciri-ciri prebiotik madu Kelulut dimana kandungan karbohidrat berjaya dikekalkan. Menariknya pertumbuhan terencat direkodkan oleh strain *laktobasilus* yang menggunakan madu rawatan HPP 600 MPa/10 minit sebagai sumber karbohidrat berdasarkan tempoh min masa gandaan (T_d) yang panjang(> 8 j). Kandungan fenolic yang tinggi disimpulkan sebagai perencat dimana korelasi yang tinggi ($r > 0.8$) dicatatkan antara kandungan fenolic dan min masa gandaan (T_d). Namun, HPP berjaya menghasilkan madu kelulut bermutu dengan kandungan fenolik dan antioksidan yang tinggi serta mengekalkan ciri-ciri prebiotik. Kesimpulannya, HPP berjaya dibuktikan sebagai cara pemprosesan madu Kelulut yang lebih baik berbanding rawatan haba dan mempunyai implikasi semasa pascatuai dalam penambahbaikan dan pengekstrakan sebatian bio-aktif.

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LIST OF SYMBOLS AND ABBREVIATIONS

AA (%)	-	Antioxidant activity in percentage
ABI	-	Agro-Biotechnology Institute
Abs	-	Absorbance reading
Abscontrol	-	Absorbance reading of the control
Abssample	-	Absorbance reading of the sample
ANOVA	-	One-way Analysis of Variance
AU	-	Absorbance unit
BI	-	Browning index
DN	-	Diastase number
DPPH	-	2,2,diphenyl2picrylhydrazyl
FAO	-	Food and Agriculture Organization of the United Nations
g	-	Gram
HHP	-	High hydrostatic pressure
HMF	-	Hydroxymethylfurfural
HPP	-	High pressure processing
HSD	-	Honestly significant difference
L	-	Litre
m	-	Meters
mm	-	Millimeters
MARDI	-	Malaysian Agricultural Research and Development Institute

min	-	Minutes
mL	-	Mililitre
MPa	-	Megapascal
nm	-	Nanometers (wavelength)
PCR	-	Polymerase chain reaction
pH	-	Decadic logarithm of acid dissociation
r	-	Correlation coefficient
RDI	-	Recommended daily intake of energy
rpm	-	Revolutions per minute (measure of the frequency of a rotation)
RSM	-	Respond surface methodology
TCD	-	Total colour difference
TDS	-	Total dissolved solid
TPC	-	Total phenolic content
TSS	-	Total soluble solid
UHP	-	Ultrahigh pressure processing
UV	-	Ultraviolet
UV Vis	-	Ultraviolet–visible spectroscopy
°C	-	degree Celsius
<	-	Less than
>	-	Greater than
±	-	Plus minus
%	-	percentage

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CHAPTER 1

INTRODUCTION

1.1 General introduction

Remarkably known for its health properties, honey has been utilized since long before the advent of modern medicine. Prominent as the oldest natural sweetener, honey has always been part and parcel of human lives. From a food supplement up to its application in medicine, honey proved to be a precious gift Mother Nature has ever provided to human kind. The unique combination of more than 181 components in honey (Alvarez-Suarez *et al.*, 2010; Bogdanov *et al.*, 2008) possess by honey of different origin have intrigued researcher all over the world to do an in-depth investigation. Ranging from its nutritional value to its medicinal properties (Ajibola, Chamunorwa, & Erlwanger, 2012), researchers are yet to discover more about the full potential of honey.

Even though many different kinds of honey from different sources have been investigated year by year, Kelulut honey, which is unique to Malaysia, is still not being investigated thoroughly. Kelulut honey which is produced by a stingless bee has been on demand in Malaysia for its proclaimed nutritional value. The production

and commercialization of Kelulut honey is still on the early stage hence more thorough studies should be done regarding all aspect of Kelulut Honey.

The constitution of honey is generally of 84% fructose and glucose with a much complex mixture of oligosaccharides. Honey has been investigated to have the ability to promote the growth of probiotics such as *Lactobacillus* and *Bifidobacterium* and suppress potentially pathogenic bacteria (Das, Datta, Mukherjee, Bose, & Ghosh, 2015; Shin & Ustunol, 2005). Oligosaccharides are neither hydrolyzed nor absorbed in the upper part of the gastrointestinal (GI) tract thus capable to act as selective growth-promoters for bacteria beneficial to the GI microflora by promoting its health (Shamala, Jyothi, & Saibaba, 2000). Honey possess the potential as another prebiotic source in which Pakistan honey, Malaysia honey and Sesame honey has supported the growth of *Lactobacillus* and *Bifidobacterium* which are the good bacteria in the GI of human being (Das *et al.*, 2015; Jan Mei, Mohd Nordin, & Norrakiah, 2010; Kamran *et al.*, 2006)

Despite the increasing number of studies on honey as prebiotic, there is yet a study on the effect of honey processing on its prebiotic potential. Conventional thermal processing in industries can be detrimental to the biological component of honey due to the unstable and thermolabile components when it is heated at 60 °C and above (Turkmen *et al.*, 2006). Thus, non-thermal approach, particularly the high-pressure processing (HPP) can be used as an alternative to maintaining the high quality of honey. Current studies of honey subjected to HPP showed an improvement in its nutritional values and quality (Fauzi *et al.*, 2013; Fauzi *et al.*, 2014; Fauzi *et al.*, 2016)

Hence, this work attempts to apply HPP as non-thermal processing to maximize the overall quality and enhance the prebiotic activity of Kelulut honey. In a way, this novel technique might be the new innovative for postharvest optimization of bioactive compound in Kelulut honey and thus has an impact on extensive usage of kelulut honey as prebiotic in food industries.

1.2 Problem statement

Recent years have seen the rise of studies on Kelulut honey. However, the information on its processing is limited and too little attention has been paid on its effect to prebiotic potential. Conventional thermal processing with a higher temperature than 60 °C in the honey industry brought about more harm to many of thermolabile components where improvement of nutritional value is very unlikely. Therefore, high-pressure processing (HPP) as the alternative processing method might prove to be of value as it is carried out without applying significant heat and preserving most compound. Despite the accretion of studies on Kelulut honey, there is no report that described the effect of HPP on Kelulut honey. By studying its effect on the overall quality that includes nutritional value and prebiotic potential, it will aid the Kelulut honey industry as well as improving Kelulut honey as prebiotic source and its diversification in the food industry.

1.3 Objectives

This research is fundamentally based on two main research questions. First and foremost, is there any significant improvement in quality (physicochemical and nutritional properties) of HPP-treated Kelulut honey as compared to conventional thermal treatment? Secondly, does the prebiotic activity of Kelulut honey enhanced by the high-pressure processing (HPP) treatment? Therefore, this study embarks on four distinct objectives as follow:

1. To investigate the changes in physicochemical properties of HPP-treated Kelulut honey as compared to conventional thermal processing.
2. To determine the changes in the nutritional properties of HPP-treated as compared to conventional thermal processing.
3. To evaluate the prebiotic potential of HPP-treated Kelulut honey as compared to conventional thermal processing tested on *Lactobacillus*.
4. To evaluate the best HPP condition for post-harvest treatment of Kelulut honey.

1.4 Scope

1) This study is on Kelulut honey processing. High-pressure processing (HPP) is the main processing method where it is carried out at 200 and 600 MPa with processing time of 5 and 10 minutes each, at ambient temperature (<38 °C).
2) Conventional thermal processing is also carried out. It is first carried out as a preliminary study at temperatures of 30, 60 and 90 °C for 10, 30 and 60 minutes each. Based on preliminary study, thermal treatment at 60 and 90 °C with time of 10 and 30 minutes is carried out for the purpose of comparison to HPP.
3) Changes in quality after respective treatment is evaluated based on physicochemical properties (pH, moisture content, total soluble solid (TSS), total solid, reducing sugar, diastase activity, free acidity and colour) and nutritional properties (antioxidant activity, total phenolic content brown pigment formation).
4) Kelulut honey processed at selected settings for each process (600 MPa/10 minutes for HPP and 60 °C/30 minutes for thermal treatments) is tested for its prebiotic potential through two strains of *Lactobacillus: L.acidophilus* and *L.brevis.* The results are evaluated in term of growth curve studies and the mean doubling time (T_d) of both strains.

CHAPTER 2

LITERATURE REVIEW

2.1 Honey: nature oldest sweetener

Honey, a natural elixir regurgitated by bees contained various benefits for humankind. Honey has been applied in human being lives since the medieval time where one of the earliest evidence dated back 8000 years ago. Its contribution is so diverse, covering aspect like nutritional food in which it promotes growth, act as a source of antioxidants, aid athletic performance, improving digestion and absorption as well as enrich children nutrition (Ajibola *et al.*, 2012). In addition, honey has a fair share in medicine development where it is useful in various aspect of medicine such as hematology, immunity, gastroenterology, ophthalmology, metabolic and cardiovascular effects, chemotherapy and wounds management, oral health as well as its antimicrobial activity (Ajibola *et al.*, 2012).

The usage of honey in human life is comprehensive regardless of age, gender, religion and transcend through civilization. Honey is regarded as the beneficial drinks in Islam and has been used in Islamic medicine since long before. There is also a complete chapter called Surah al-Nahl carrying the meaning of Honey Bee in the Holy Quran which emphasizes the special potential and benefits honey possess (Ajibola *et al.*, 2012; Etera-Oskouei & Najafi, 2013). Honey has also made its way in various civilization and cultures such as ancient Greece, ancient Egypt and Indian Ayurveda in which it is used to treat those with frail digestion, cough, eye disease, baldness and many others (Eteraf-Oskouei & Najafi, 2013). This hence shows honey

is very valuable and has many more potentials to be unveiled in today world where advanced technology is easily accessed.

2.1.1 Honey: general composition and valuable properties

Honey is a liquid or an elixir that is regurgitated by bees. It can be classified into two categories with two variations. The first category is blossom honey and the second category is honeydew honey. Blossom honey is produced primarily from the nectar of flowers whereas honeydew honey is produced predominantly from the excretion of plant or excretions of plant-sucking insects (Hemiptera) on the living parts of the plants which the bees ingest and secrete it back. These two categories have two subsequent variations where it can either be monofloral or multi-floral honey. Monofloral honey as the name indicates is sourced from a single flower. Honey from this variation is produced when bees predominantly forage for a single flower source. On the other hand, multi-floral honey is produced from multiple flower sources with none of the sources tops over the others. These variations are dependent on geographical factor (proportion of flowers) as well as the time of harvest. All these variations hence make each honey have a distinct taste and aroma from one another though sometimes it is regurgitated by the bees from the same family.

The variation of botanical sources, geographical origin as well as way of handling and storing consequently influence the composition of honey (Gheldof, 2002; Turkmen *et al.*, 2006). Honey is said to compose of approximately 181 to 200 different components working harmoniously and make up the uniqueness of honey. Generally, fructose and glucose dominate the content of honey by 38% and 31% respectively (Alvarez-Suarez *et al.*, 2010). Vitamins, amino acids, mineral, protein and enzymes are the other components that are generally found in honey (Alvarez-Suarez *et al.*, 2010; Eteraf-Oskouei & Najafi, 2013). Besides the major component, there are numerous of minor components such as Maillard reaction product, phenolic acids, flavonoids, ascorbic acid, tocopherols, catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), glucose oxidase and catalase (Alvarez-Suarez *et al.*, 2010; Eteraf-Oskouei & Najafi, 2013). These minor products are working in sync to give out the antioxidant properties possess by honey. The high antioxidant content

in honey, flavonoids, amino acids and phenolic acids may reduce the risk of some forms of cancer, heart disease, strokes, and cataracts and may slow the aging (Ajibola *et al.*, 2012; Khalil & Sulaiman, 2010). The aforementioned components are what generally composed in honey but the ratio in a different type of honey is somewhat very distinct. An exact composition standard applicable for all types of honey is so far unattainable due to its nature that is highly dependent on the geographical origin. The general composition of honey is shown in Table 2.1 whereas the different biological effects of honey characteristics with respect to its nutritional, physical, microbiological and medicinal aspects are summarized and presented in Table 2.2.

Table 2.1 General Composition of honey (data in g/100g) Adapted from (Ajibola *et al.*, 2012; Alvarez-Suarez *et al.*, 2010; Eteraf-Oskouei & Najafi,2013)

Component	**The range of percentage (%)**
Water	17.1
Total sugar	79.7-80.5
Monosaccharides	
fructose	31.8-38.2
Glucose	26.1-31.1
Disaccharides	
sucrose	0.5 - 0.7
Others	4.0 - 5.0
Trisaccharides	
oligosaccharides	3.1-10.1
Erlose	0.1-0.8
melezitose	< 0.1-0.4
Others	0.4-3.0
Minerals	0.2-0.9
Amino acids, protein	0.3-0.6
pH value	3.9-5.2
Free acid as gluconic	0.43
Lactone as gluconolactone	0.14
Total acid as gluconic	0.57
Ash	0.169
Nitrogen	0.041
Vitamin C	0.0005
Calcium	0.006
Iron	0.00042
Magnesium	0.002
Phosphorus	0.004
Potassium	0.052
Sodium	0.004
Zinc	0.00022

Table 2.2 Summaries of honey characteristics with respect to its nutritional, physical, microbiological and medicinal properties

Characteristics	**Remarks**	**References**
Nutritional Aspect		
Carbohydrates Composition	Constitute 95% of dry weight.	Alvarez-Suarez *et al.*, (2010); Eteraf-Oskouei & Najafi, (2013)
	Carbohydrates in honey consist of a highly complex mixture of sugars with most are in the instantly digestible form in the small intestine.	
	Instead of forming in nectar, Many of these sugars are formed during the ripening and storage effects of bee enzymes along with the acids of honey	
	It is an effective carbohydrate source for athletes before and after resistance training and during endurance exercise	
Vitamins/Minerals and trace elements	These characteristics depend on the botanical and geological origin.	Alvarez-Suarez *et al.*, (2010); Saha, (2015)
	Trace elements play a key role in the biomedical activities associated with honey as they possess a multitude of known and unknown biological functions.	
	In particular, chromium, manganese and selenium are vital for 1-15 years old children.	
	Sulphur, boron, cobalt, fluoride, iodide, molybdenum and silicon are the elements that have potential to give significant impact in human nutrition	
	In general, the amount of vitamins and minerals is small but vitamins such as phyllochinon (K), thiamin (B1), riboflavin (B2), pyridoxine (B6) and niacin are reported in honey.	
Aroma compounds	Known as a ‘‘fingerprint’’ of the product as the aroma profile could be used to determine its origin.	Alvarez-Suarez *et al.*, (2010)
	Aroma-building compounds are dependent on the botanical origin thus vary in composition in different types of honey.	
	Exist at very low concentrations as complex mixtures of volatile components of different functionality and relatively low molecular weight	
	The compound characteristic identified for citrus honey was methyl anthranilate	
Polyphenol	Polyphenols such as quercetin, acacetin, caffeic acid phenethyl ester (CAPE), kaempferol, and galangin in honey was reported to have a likely potential as pharmaceutical drugs in the treatment of cardiovascular diseases.	Khalil & Sulaiman, (2010); Alvarez-Suarez *et al.*, (2010)
	Flavonoid is the main polyphenols, with a varied content between 60 and 460 μg/100g of honey.	
	The three main phenolic, namely benzoic acid, cinnamic acids and flavonoids are distributed verily thus showing different profiles in honey from different floral origins, with flavonoids being the most common in.	

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