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A Comparative Study on Thermal and Non-thermal Processing of Kelulut Honey

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Abstract: Recent years have seen the various studies conducted on the kelulut honey based on their physicochemical and nutritional properties. The main concern in production of kelulut honey in maintaining the quality especially when it is being treated. Previous studies have shown that conventional thermal processing that comprises two phases of heating which are liquefaction and pasteurization will minimize the quality of kelulut honey in terms of its physicochemical and nutritional properties due to its heat sensitivity material criteria. In addition, the reduction in quality of honey due to the unsteady components, breakdown of vitamins and damage of enzymes when it heated at more than 60℃ in thermal processing. Hence, a systematic review is carried out to identify a suitable heat treatment for kelulut honey as compared to conventional thermal processing in treating honey. The search strategy was established in three databases (Google Scholar, PubMed and ScienceDirect) with the search keywords "("Honey", "Kelulut", "stingless bee", "Trigona", "Melipon", "Microwave heating", "Double boiling", "HPP" or "High pressure processing" and "Ultrasound")". The data selection strategy was showed using PRISMA guidelines. A total of 311 journal papers were referred to complete this review, however, only 10 articles were highly considered specifically on the process of kelulut honey, where microwave heating, double boiling and high pressure processing (three articles respectively). Whereas, only two articles on analysis of ultrasound were found. From this literature review, it found that HPP provide higher benefit in improving physicochemical and nutritional properties of kelulut honey although it produces a lower change in colour if compared with other treatment. It can be concluded that HPP is the most potential in increasing the quality of kelulut honey followed by double boiling, ultrasound and microwave heating.

Keywords: Kelulut honey, microwave heating, double boiling, high pressure processing, ultrasound

1. Introduction

In Malaysia, stingless bee honey known as kelulut honey. It can be classified into two types of species which are Melipona and Trigona [6]. Kelulut honey is the natural food with large amount of nutritional and beneficial value. The colour of honey is clearer and provide combination of sour and sweet flavours [16]. In the past three decades, honey quality was studied and the processing of honey played an important role toward its physicochemical properties. The honey has been studied and compared as it has different botanical and geographical origins. Generally, honey is heated for preventing microorganism and crystallization in processing plants. Conventional thermal processing is a suitable way in preventing fermentation [8]. However, high temperature that comprises two phases of heating which are liquefaction and pasteurization exposure will minimize the quality and biological properties of honey. In addition, the reduction in quality of honey due to the unsteady components, breakdown of vitamins and damage of the enzymes when it is heated at more than 60℃ in conventional thermal processing [8].

The research of kelulut honey processing is still unexplored. The composition of stingless bee honey may increase the immune system and provide cell functions in erythrocytes which connected with cell antiseptic, antimicrobial,

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anticancer, anti-inflammatory and wound therapeutic properties [16]. However, these valuable properties will be affected by their conventional heat treatment processing. To improve the honey quality, advanced technologies and innovations need to be developed in honey processing with the conventional thermal method. This study is focused by the current awareness, which is to identify the technologies in kelulut honey processing for better improvement in its quality. The quality of kelulut honey has been abandoned due to the lack of systematic scientific studies for honey processing [16]. Thus, kelulut honey in the world market became incomplete because of lack of standard, lower shelf life and lower industrial production [13]. Recently, microwave heating, double boiling, high pressure processing (HPP) and ultrasound are the processing used in treated kelulut honey. Microwave heating and double boiling classified as thermal processing meanwhile HPP and ultrasound classified as non-thermal processing. These different treatments may affect the taste, texture, appearance or nutritional value of kelulut honey. Yet, there is no report and investigation has been conducted on comparative study on thermal and non-thermal processing of kelulut honey. Therefore, the current study is undertaken to investigate the effect of different treatments (thermal and non-thermal) on the physicochemical and nutritional properties of kelulut honey.

2. Materials and Methods

2.1 Search Strategy

A literature search was performed that cover the period from 2000 until 2020 by using three major databases, which are Google Scholar, PubMed and ScienceDirect. The compatible references were re-evaluated for confirmation of the search string. Moreover, the relevant references were also involved as an extra source of literature reports. All the search databases were exported into Mendeley software to check and remove duplicates of searched data in each database. “Honey”, “Kelulut”, “stingless bee”, “Trigona” and “Melipona” were used as keywords to determine the article of kelulut honey. In addition, “Microwave heating”, “Double boiling”, “HPP” or “High pressure processing” and “Ultrasound” were added along with the previous stated keywords in order to focus the searches for the kelulut honey processing.

2.2 Inclusion Criteria

Stingless bee is also known as Kelulut, Trigona and Melipona that be described in the literature review. In this review, the studies to investigate the quality of kelulut honey were involved. From the databases, only kelulut honey processing by using microwave, double boiling, high pressure processing (HPP) and ultrasound were included in this study. Studies published in English was taken into consideration.

2.3 Exclusion Criteria

The papers that have not been written in English were not included in the study. The honeybee or other species that not related with stingless bee were excluded from the study. The studies of kelulut honey processing except microwave heating, double boiling, HPP and ultrasound were also excluded for this review.

2.4 Study Selection

Major literature search was performed by authors. At first, Mendeley software filtered out all the duplicate articles and monitored by hand search to ensure there is no identical articles were included. The possible papers were picked by selection of the title, abstract and recovery of the overall papers from the database search. Meanwhile, the irrelevant articles were remove based on the inclusion and exclusion standards. Then, the chosen articles were downloaded and been evaluated.

2.5 Data Organization and Reporting

The data obtain from each study were organized in a table that included the author’s name, year of publication, title, type of heat treatment used, type of stingless bee species and outcomes. The studies were stated based to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [5]. This method involved the construction of an outline based on the number of articles identified, included, excluded and the reasons for exclusions throughout the phases [16]. It provides a flowchart to demonstrate the searching strategy until the evaluation process.

2.6 Descriptive of Selected Studies

Figure 2.1 shows the PRISMA flow diagram of search strategy and selection process for kelulut honey processing. A total of 311 were identified after searching with three different databases which are Google Scholar, PubMed and ScienceDirect. After removal of duplicates, about 276 articles were separated. Then, 127 articles were removed because not focusing on kelulut honey processing and 149 articles were reassessed again. Finally, 121 articles that not written in English language and not focusing on microwave heating, double boiling, high pressure processing and ultrasound were

removed and only 10 of the reports were choose for this review. All the 10 reports were identified and further analysis based on the category of kelulut honey form differences stingless bee species and outcomes. The summarized were tabulated on Table 2.1, 2.2, 2.3 and 2.4.

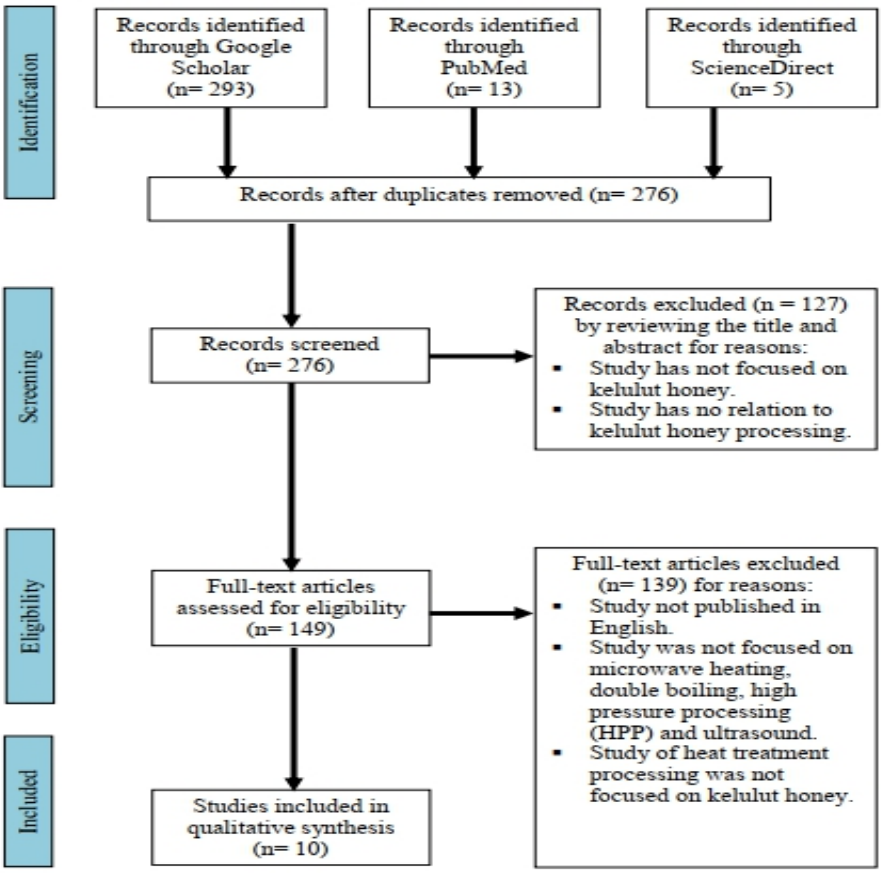

Figure 2.1 - PRISMA flow diagram of search strategy and selection process for kelulut honey processing [16]

Table 2.1 - Microwave heating of kelulut honey processing

Authors and Year	Types	Method	Outcomes
Ramli et al. [11]	*Trigona spp*	Combination of low temperature vacuum drying with microwave.	The radiation of microwave may reduce the nutritional content of stingless bee honey. It may affect the original quality, aroma and taste of honey.
Lemos et al. [7]	*Scaptotrigona sp*., *Melipona fasciculate*, *Melipons flavolineata* and *Apis mellifera*	Microwave oven used to digest the sample of honey with diluted nitric acid and hydrogen peroxide.	*Scaptotrigona sp*. species provide large amounts of minerals source
Yegge et al. [17]	*Heterotrigona itama spp*	In microwave treatment, the power level of 20, 60 and 100 for 10s, 15s, 30s and 60s that performed in a micro convective oven.	A significant in decreases moisture content and increasing phenolic content at higher power level (60PL) and longer treatment time (60s) by using microwave treatment.

Table 2.2 - Double boiling of kelulut honey processing

Authors and Year	Types	Method	Outcomes
Akhmazillah et al. [1]	*Trigona spp*	The double boiling treatment was performed at 30℃, 60℃ and 90℃ for 10, 30 and 60 minutes using a double boiler.	The optimum conditions of double boiling treatment at 60℃ than untreated honey. At 90℃, the brown pigment significantly increases with higher heating temperature. No significant changes of moisture content, total soluble solid and total solid in overall temperature.
Rahman et al. [9]	*Trigona spp*	The honey samples were heated at different temperature (30-60℃) for different treatment time (10-60 min) with a double boiler.	The optimum conditions of double boiling treatment at 60℃ for 34.69 min due to lower treatment time and below boiling temperature. The lowest moisture content at 27.86% with highest total solids, total soluble solid and antioxidant activity at 72.14%, 70.75 °Brix and 65.44% was found in those conditions.
Rahman [10]	*Trigona spp*	Three different samples of kelulut honey from types of foods consumed by bees (coconut, multifloral and wild flower) were heated to an optimized condition at 60℃ for 35 minutes with double boiler.	The double boiling treatment increase the total phenolic content and brown pigment of kelulut C (coconut) and kelulut W (wild flower). The treatment improves the physicochemical properties of three types of kelulut honey compared to untreated samples in a four weeks storage.

Table 2.3 - High pressure processing (HPP) of kelulut honey processing

Authors and Year	Types	Method	Outcomes
Razali [12]	*Trigona spp*	HPP was conducted at two different pressures (200 and 600 MPa) with two different time (5 and 10 minutes).	HPP provide better retention of total acidity, reducing sugar, total sugar and diastase activity. Increase the antioxidant activity for 3% and total phenolic content for 47.2% at 600MPa/10 minutes.
Razali et al. [13]	*Trigona spp*	HPP was conducted at two different pressures (200 and 600 MPa) with two different time (5 and 10 minutes).	The uses of HPP on kelulut honey provide a better retention of antioxidant activity by 3% at 600MPa/10 minutes. There is no significant changes ($p>0.05$) on diastase activity.
Razali et al. [14]	*Trigona spp*	Kelulut honey samples was exposed to HPP at 600 MPa at 10 minutes.	Maintain the amounts of carbohydrates and increasing the total phenolic content by 47.2%. Increasing the probiotic potential against *Lactobacillus* strain.

Table 2.4 - Ultrasound of kelulut honey processing

Authors and Year	Types	Method	Outcomes
Akhmazillah et al. [1]	*Trigona spp*	Indirect sonication processing was performed at 30℃, 60℃ and 80℃ for 10, 30 and 60 minutes with a constant frequency of 37 kHz using an Elmasonic S180H ultrasonic bath.	No significant difference of moisture content for overall processing temperature. Highest pH value was obtained from treatment temperature at 30℃. The brown pigment and antioxidant activity will increase at higher temperature.
Chong et al. [2]	*Trigona spp*	Thermosonication treatment of honey was performed at 45.0, 55.0, 67.5, 80.0 and 90.0℃.	Thermosonication improve the quality in terms of water activity, moisture content, color intensity, viscosity, total phenolic content and radical scavenging activity.

3. Results and Discussion

3.1 Search Strategy

3.1.1 Thermal of Kelulut Honey Processing

3.1.1.1 Microwave heating

Yegge et al. [17] studied the effect of microwave heating of kelulut honey in minimize the moisture content. The reduction in moisture content of kelulut honey can occurred with high power level (60 and 100PL) and longer treatment time. The study also indicated a higher of phenolic content for 43% at power level of 60 for 60 minutes. The increment of brown pigment was significantly occurred at all power level not including the samples treated for both 20PL and 60PL in longer treatment time (30 and 60 min). The increment of total soluble solid (TSS) and total solid at power level of 60 and 100 for all treatment times. However, the microwave treatment showed reduction in antioxidant activity and pH.

Ramli et al. [11] conducted studies on microwave with lower temperature vacuum drying on kelulut honey. The experiments may increase the honey dewatering rate for about $\eta_D = 0.25\%$ per minute. However, the radiation exposure by the microwave reduces the quality, aroma and taste of the honey. Lemos et al. [7] studied the physicochemical composition and inorganic constitutes with four different stingless bee species in Amazon which are *Scaptotrigona* sp., *Melipona fasciculata*, *Melipona flavolineata* and *Apis mellifera*. The study performed in a microwave oven with honey samples by using diluted nitric acid and hydrogen peroxide. The honeys from Scaptotrigona sp was found contained high amount of minerals (Ca, Cu, K, Mg). Among all the species, the reduction was occurred in soluble solids and moisture content with microwave treatment.

3.1.1.2 Double Boiling

Double boiling techniques in kelulut honey treatment is still underexplored. According to Rahman et al. [9], the highest reduction of moisture content by 4.22% occurred when treated kelulut honey with double boiled at 60 °C for 10 minutes compare to untreated samples. A larger value of total soluble solids (TSS) and total solids showed high sugar content in honey which contribute large amounts of antimicrobial. The highest brown pigment was occurred at 60℃ for treatment time (10 minutes). A higher brown pigment showed in kelulut honey may showed a higher antioxidant property. The brown pigment of kelulut honey affected by high temperature but not treatment time. It was indicated with the results obtained which lowest brown pigment occurred at temperature (30℃ and 60℃) with same time treatment (10 min). The highest antioxidant produced at 60 °C for 35 min at treatment temperature. In addition, antioxidant activity provides a strong relation with physical properties (pH, color, electrical conductivity and TSS) of honey.

Meanwhile, another studied was performed by Akhmazillah et al. [1] found that the higher pH values of double kelulut occurred at 30℃ and 60℃ than the untreated kelulut honey. Meanwhile, pH values at 90℃ showed lower than untreated. After that, the treated of kelulut honey with double boiling was indicated the slightly changes on the moisture content, total soluble solid and total solids compare to untreated honey. The brown pigment of kelulut honey was higher when treated with double boiled at temperature of 80℃ for 60 minutes as shown. The rises of treatment temperature and time will increase the antioxidant activity of kelulut honey while treated with double boiled. However, the research found that the reduction antioxidant activity double boiled than untreated.

3.1.2 Non-thermal of Kelulut Honey Processing

3.1.2.1 High Pressure Processing

In the literature review, high pressure processing (HPP) was performed at the same condition of pressures (200 and 600MPa) with same treatment time at 5 and 10 minutes [13, 14]. Whereas, Razali [12] was performed study on the HPP treatment of kelulut honey with *Lactobacillus* strains. Razali et al. [13] studied indicate that HPP may improve the antioxidant properties of kelulut honey. It can be showed with increase the antioxidant activity at 600 MPa with 10 minutes processing. Another studied also shown the increment of antioxidant at 600MPa/10 minutes by 3.0% [12].

Then, HPP processing will increase the diastase activity of kelulut honey. The results showed the increment of diastase activity except for 200MPa with 5 minutes. Therefore, it demonstrated the capability of HPP to produce a fresh like quality of food product. Razali et al. [14] also identified the changes in colour with HPP processing. Therefore, it showed the limitation of HPP processing towards colour of kelulut honey. In addition, the kelulut honey processing with HPP was improved the quality of honey with increase the total phenolic content (TPC) and better prebiotic characteristics. It can be showed with limited experimental in *Lactobacillus* strains grown with HPP treated at 600MPa/10 minutes by longer doubling time. Razali [12] was concluded that optimum condition to maintain the kelulut honey for prebiotic potential and increasing nutritional at treatment pressure of 600MPa for 10 minutes.

3.1.2.2 Ultrasound

There are limited studied on ultrasound with kelulut honey in physicochemical and nutritional characteristics. Akhmazillah et al. [1] conducted studies on application of indirect ultrasound as an alternative to the conventional treatment. From the research, the highest pH values occurred in 30℃ for 30 minutes and the results was different with all treatments at temperature of 60℃ and 90℃. The results of total solids and total soluble solids with ultrasound become fluctuated in increasing treatment time. The higher processing time and temperature may increase the brown pigment and antioxidant activity of kelulut honey.

Whereas, another studied of ultrasound was conducted by Chong et al [2] to improve the storage quality of raw kelulut honey. The combination of heat and ultrasound was known as thermosonication techniques. The application of thermosonication can minimize the moisture content due to the high mass transfer by collapsing microbubbles. The techniques also increase the colour intensity at higher temperatures and longer treatment time. Interestingly, the thermosonication was found to increase the phenolic content of kelulut honey.

3.2 Discussions

The analysis of physicochemical for honey is important in the global trade which play important role in a growing variety of food products. pH is crucial which affects the texture, stability and shelf life of kelulut honey [10]. A high amount of moisture content may lead fermentation risk to kelulut honey [17]. The moisture content is related with total solids and total soluble solid (TSS) which contribute in the quality of honey. A larger value of total solids and TSS showed high sugar content in honey which contribute large amounts of antimicrobial [9]. Meanwhile, a higher brown pigment showed in kelulut honey may showed a higher antioxidant property. Antioxidant also significant features in nutritional properties of honey [3].

This systematic review recognizes 10 reports on kelulut honey processing with four different types of heat treatment which are microwave heating, double boiling, high pressure processing (HPP) and ultrasound. In conventional honey processing, the application of heat is needed to reduce the time and dissolve large sugar granules at a temperature of more than 50℃ until 77℃ [15]. Recent studies have shown that thermal treatment will minimize the nutritional value, texture and taste of honey [4]. In the review, the quality of honey which are physicochemical properties (pH, moisture content, total solids, total soluble solids (TSS), brown pigment and antioxidant activity were investigated by using different heat treatment. The summary of thermal and non-thermal processing of kelulut honey in terms of physicochemical and nutritional properties shows in Table 3.1.

Table 3.1 - The summary of thermal and non-thermal processing of kelulut honey in terms of physicochemical and nutritional properties

	Advantages	Disadvantages
Thermal processing of kelulut honey		
Microwave heating	• Reduce moisture content • Increases phenolic content • Increases brown pigment • Increase total soluble solid (TSS) and total solid	• Reduce antioxidant activity • Reduce pH • Radiation exposure will reduce the quality and taste of honey
Double boiling	• Reduce moisture content • Increases pH • Increases brown pigment • Increase total soluble solid (TSS) and total solid	• Reduce antioxidant activity
Non-thermal processing of kelulut honey		
High pressure processing (HPP)	• Reduce moisture content • Increases antioxidant activity • Increase phenolic content	• Low changes in colour (brown pigment)

	• Increase diastase activity • Better prebiotic characteristics	
Ultrasound	• Reduce moisture content • Increases pH • Increase brown pigment • Increase antioxidant activity	• Slight changes in total solid and TSS • Lack effective in removing microorganisms

4. Conclusion

As a conclusion, this study has identified microwave heating, double boiling, high pressure processing (HPP) and ultrasound via method of PRISMA guidelines with three different databases (Google Scholar, ScienceDirect and PubMed). From the literature review, it found that high pressure processing (HPP) provides higher benefit in improving physicochemical and nutritional properties of kelulut honey if compared with other treatment. Thus, HPP is the most potential in increasing the quality of kelulut honey followed by double boiling, ultrasound and microwave heating. This study can be further research with various database in wider range of year, more comparison of thermal and non-thermal treatment in kelulut honey processing or different types of honey in order to obtain maximum efficiency of data and result of physicochemical and nutritional properties.

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