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EFFECT OF HEATING ON FLAVONOID CONCENTRATION IN HONEY HARVESTED FROM ACROSS INDONESIAN ARCHIPELAGO

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Abstract

Indonesia is an archipelagic country with a vast area. As one of the commodities produced in Indonesia, honey has very different characteristics between regions. The quality of honey between different regions is very different, so it is interesting to study. In addition, the quality and flavonoid content of honey can still be improved by the heating process. This study aimed to determine the effect of heating honey on flavonoid levels. Heating was carried out at a temperature of 65°C and 121°C. Flavonoid concentration in honey samples was measured based on AlCl3 methods. It turns out that heating on honey can increase flavonoid levels from *Apis dorsata, A. mellifera*, and stingless bees. Variations in flavonoid levels were also shown due to the influence of the location where the honey was taken. The highest total flavonoid content from *A. dorsata* was obtained from Flores honey, A. mellifera honey obtained from mango honey, and stingless bee honey from Banten II honey with 1224.63 996.71, and 1109.77 mg QE/L, respectively. The percentage increase in the effect of heating at 121 °C from the highest total flavonoid content compared to not heating was 259.97 %, 267.94 %, and 260,53 %. The highest percentage increase was experienced by stingless bee honey from Banten 2 (260.53 %), *A. dorsata* honey from Rupat Island (624.78 %), and A. mellifera honey from Acacia (702.63%). These results indicate that heating carried out on all types of honey in Indonesia can increase flavonoid levels, and there is a difference between the location of origin and the type of bee.

Keywords: *Apis dorsata, Apis mellifera, Stingless bee, flavonoid, Indonesian honey*

Introduction

Public health is still a problem that the government pays attention to today. The current pattern of human life can cause various problems that result in decreased levels of health. Unhealthy lifestyles, such as irregular mealtimes, lack of fruit and vegetable consumption, physical activity, excessive consumption of alcoholic beverages and cigarettes, which are increasingly loved by the community, are the leading causes of degenerative diseases. The data [1] shows that almost 41 million people die (about 71%) every year due to degenerative diseases. The occurrence of degenerative diseases is influenced by oxidative stress in the body, which is triggered by free radicals [2].

Free radicals are highly reactive and unstable molecules in the body. Free radicals have one or more unpaired electrons in their outer layer. These highly reactive electrons stabilize themselves by reacting to their surroundings to find electron pairs. This reaction can occur simultaneously and continuously, namely the tendency to attract electrons and convert a molecule into a new free radical so that a chain reaction occurs until a stable condition is reached. This chain reaction must be stopped as soon as possible to prevent degenerative diseases, such as cancer, heart failure, cataracts, and others [3]. The process of free radical stabilization only stops if the free radicals are quenched with antioxidants.

Antioxidants can counteract free radicals by donating electrons to free radicals to become stable and inhibit free radical chain reactions. Antioxidants can be obtained from within the body or outside the body or food. Many local natural ingredients contain antioxidants with various active substances, including vitamins C, E, pro-vitamin A, organosulfur, -tocopherol, flavonoids, thymoquinone, and statins, niacin, phycocyanin, and others [4]. Natural ingredients that can be used as a source of antioxidants are honey [5].

Honey is a natural liquid that has a thick texture and a sweet taste. Honey is produced by bees (Apis sp.) from plant flower extracts (floral nectar) or other parts of plants (extra floral). There are various types of honey in Indonesia, such as randu honey, rambutan honey, multiflora honey (Apis dorsata, wild bee), and stingless bee honey. The honey produced by bees is still natural because it is obtained directly from the forest.

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Honey is a commodity favored by the community because it has many benefits. The main nutritional content of honey is a variety of carbohydrate compounds such as fructose, glucose, sucrose, and carbohydrate dextrins. According to [6] honey has antimicrobial, antiviral, antiparasitic, anti-inflammatory, antimutagenic, immunosuppressive, and anticancer activities. Honey has also been shown to be rich in phenolic compounds and flavonoids, generally known as antioxidant compounds. According to [7] honey's physicochemical properties and color will also change after heating. Therefore, changes in flavonoid levels will be studied that occur in honey due to heating.

Methods

The samples used in this study were four variants of Stingless bee (Banten I, Bangka Island, Banten II, and Klaten), *Apis dorsata* (Flores, Rupat Island, Sentarum Lake, and Siak), and *A. mellifera* (Mango, Acacia, Hairy Fruit, and Coffee). The chemicals consist of $AlCl_3$ and sodium acetate.

Research procedure

2.1 Sample Preparation

Honey samples were collected from several regions in Indonesia. Honey was stored in 1 L jerry cans and at room temperature after harvesting until analysis. Samples were treated at room temperature, 65 ºC, and 121ºC for 5 minutes.

2.2 Determination of Total Flavonoid Levels [8],[9],[10].

A total of 20 mg of the sample was weighed and dissolved in 10 mL of ethanol, then centrifuged to obtain a concentration of 2000 ppm. A total of 0.5 mL of the sample was added with 0.1 mL of 10% Aluminum (III) chloride, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. After being incubated for 30 minutes. The absorbance was measured using a UV-Vis spectrophotometer at the maximum length calculated using the linear regression equation of the previously measured quercetin calibration curve. The total flavonoid content was expressed as the mg of quercetin equivalent per gram of extract. The test was carried out with three repetitions.

Results

Total flavonoid content was measured based on the presence of quercetin in honey because its value was mg equivalent per 1 L of honey (mg QE/L). The results of the calculation of total flavonoids and the percentage increase in flavonoid levels due to heating are presented in Table 1. In addition, the results of the calculation of total flavonoid levels are presented in Figure 1 for *Apis dorsata*, Figure 2 for *Apis mellifera* honey, and Figure 3 for stingless bee honey.

Discussion

Honey is a product in the form of a liquid produced by honeybees that resembles a syrup [11]. Honey contains at least 200 compounds consisting primarily of sugar and water. Other compounds in honey include protein (in the form of enzymes), organic acids, vitamins, especially vitamins B6, minerals such as calcium, iron, and zinc [12]. In Indonesia, the various types of honey include honey from *Apis dorsata, A. mellifera*, and Stingless bees [11]. The production of stingless bee honey is less than other kinds of honey [13].

Stingless bee honey has long been traditionally consumed in Malay, Arabic, Persian, Indian and Chinese cultures because it treats various diseases [14]. Stingless bee honey has a sourer taste than other kinds of honey. The sour taste of stingless bee honey indicates the high content of antioxidants. The taste is related to the high vitamin C in stingless bee honey, an antioxidant agent [15]. The aroma produced by stingless bee honey is slightly sour, but this depends on the fruit season when the stingless bee sucks nectar from flowers [13]. The color of stingless bee honey compared to honey from other bees tends to be clearer [16]. The content of stingless bee honey compared to honey from the Apis genus is more diverse. The minerals contained in stingless bee honey include sodium, calcium, magnesium, aluminum, iron, phosphorus, potassium, and sulfur. Stingless bee honey also contains several vitamins, namely, vitamins B1, B2, B3, B5, vitamin C, and vitamin K. Stingless bee honey also contains several enzymes such as diastase and glucose oxidase peroxidase, and lipase [11].

Stingless bee honey is helpful as an antioxidant, anti-inflammatory, and antimicrobial due to the high phenolic content in honey. Research conducted by [17] [18] showed that the content of phenolic compounds in stingless bee honey could reduce the production of reactive oxygen species.

Wild bee honey is a honey produced by Apis dorsata. Wild bee honey is also called multiflora honey because it is made from various plant flowers. Generally, forest honey is blackish-brown in color. This is because forest honey contains many minerals, enzymes, and various other valuable

substances, which are richer in contents when compared to other types of honey that are lighter in color. Honey produced by Apis dorsata bees, also known as forest honey, is the most significant contributor to honey production in Indonesia. Forest bee honey has a higher price because collecting honey by forest bees occurs naturally. Honey production depends on the season and the composition of the colony. Previous research has shown that Apis dorsata honey is rich in bioactive compounds, so that honey has antioxidant activity and various other benefits of biological activity [19]. Research conducted by [20] showed that Apis dorsata honey had a higher concentration of phenolic compounds, flavonoids, DPPH, and FRAP values than A. cerana honey and A. melifera honey, indicating high antioxidant capacity. The compounds contained in honey can eradicate free radicals, reduce metal ions, and are protective against oxidative stress. The presence of phenolic compounds in honey is an essential contributor to the antioxidant capacity of honey. Research [8] showed that Apis dorsata honey contained 57.5 g GAE/g total phenolic and 766.5 g QE/g total flavonoid. Total phenolic and total flavonoids can be used to determine the potential of honey as a source of antioxidants.

The composition of honey in each monofloral honey-producing plant can be different, especially from the origin of the nectar flora and the geographical conditions of the honey, so that the quality of the honey produced will also be different [21]. In general, natural honey consists of 75% monosaccharide sugar (31% glucose and 38% fructose), 10-15% disaccharide sugar (mainly sucrose and maltose), as well as a mixture of enzymes, minerals, vitamins, amino acids, flavonoids and phenolic compounds that contribute the remaining percentage [22]. Honey from Apis mellifera has about 22 sugars with fructose and glucose as the main sugar content. The sugar content obtained in this honey can be obtained by 10.5%, with a higher fructose content than glucose [23] [24]. According to the statement of [7] and [25]. the physicochemical properties and colour of honey change during heating. By looking at the data in Table 1, honey will change its flavonoid content after heating, thereby changing the ability to become smaller or larger in antioxidant value due to heat treatment [26] , [7], [25]. The total flavonoid content of honey changed due to heat treatment, especially in the heating process at 121°C [7] and [26]. However, the changes are the increasing levels of honey flavonoids, and some even increase to more than 700% (Table 1).

Phenol compounds in the form of flavonoid compounds in honey consist of various structures. The highest compound is quercetin, so that the measurement uses the equivalent of quercetin [27], while according to [28] the main phenolic content is rutin. In this determination of flavonoids, use the common equivalent, and it is reported as the common equivalent. According to [29] that there is a positive relationship between honey colour and flavonoid content. The same opinion was also expressed by [27], [30], [31], [32]. According to [33] the components contained in honey depend on the type of bee and the location of origin (geographical location). Therefore, environmental influence in the form of climate and environmental conditions such as humidity, temperature, and soil type where the vegetation grows, and the type of vegetation plays an important role in the difference in the quality of honey. The content of flavonoids and pigments derived from these compounds is also directly related to the colour of honey and its different shades [27], [28]. According to [34], in monofloral honey, the main component is flavonoids which reach 42% of the total phenolics. However, floral and geographic origin are decisive in the flavonoid profile in honey [35]. The origin of the flower or the nectar source is a considerable influence in distinguishing honey, while storage and handling of honey have little role in honey quality [36].

Conclusion

The highest total flavonoid content from *Apis dorsata* was obtained from Flores honey, Mellifera honey obtained from Mango honey, and stingless bee honey from Banten II honey with values of 1224.63 996.71, and 1109.77 mg QE/L, respectively. The percentage increase in the effect of heating at 121°C from the highest total flavonoid content compared to not heating was 259.97%, 267.94%, and 260.53%. The highest percentage increase was experienced by stingless bee honey from Banten 2 (260.53%), *A. dorsata* honey from Rupat Island (624.78%), and *A. mellifera* honey from Acacia (702.63%). These results indicate that heating carried out on all types of honey in Indonesia can increase flavonoid levels, and there is a difference between the location of origin and the type of bee.

Conflicts of interests

The authors declare that they have no conflicts of interest.

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Table 1 Total Flavonoid Content of Honey due to heating

Honey Sample	Total Flavonoid (mg QE/L), Temperature			percentage increase (%)	
	25 °C	65 °C	121 °C	25 to 65	25 to 121
Stingless bee					
Banten II	425,972±12,81	903,241±25,75	1109,77±124,87	212,04	260,53
Bangka Island	263,703±41,12	458,842±55,18	603,15±42,20	173,10	228,72
Banten I	261,120±28,45	397,407±35,42	523,70±30,91	152,19	200,56
Klaten	231,250±25,51	361,435±24,65	494,40±31,82	156,30	213,79
Apis dorsata					
Flores	471,06±107,77	988,19±193,09	1224,63±81,54	209,78	259,97
Rupat Island	157,82±85,36	384,26±185,44	583,43±11,23	243,48	624,78
Sentarum Lake	155,46±45,88	368,94±38,20	255,00±17,27	237,32	375,29
Siak	135,56±32,29	236,39±14,70	986,02±36,43	174,38	188,11
Apis mellifera					
Mango	371,99±28,40	644,54±26,41	996,71±32,49	173,27	267,94
Acacia	99,35±72,53	395,37±27,91	698,06±13,64	397,96	702,63
Rambutan	283,15±13,32	231,81±20,14	307,64±15,62	81,87	108,65
Coffee	119,81±8,29	150,28±15,09	161,48±53,78	125,43	134,78

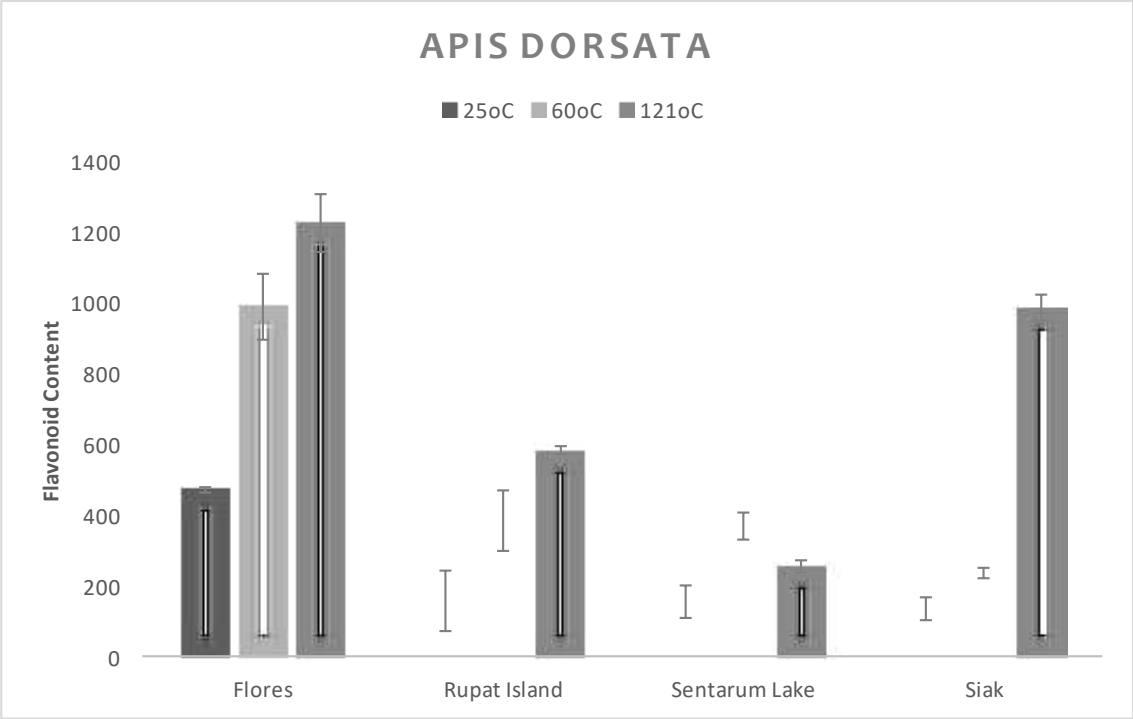

Fig 1. Total flavonoid content of honey due to heating from Apis dorsata honey

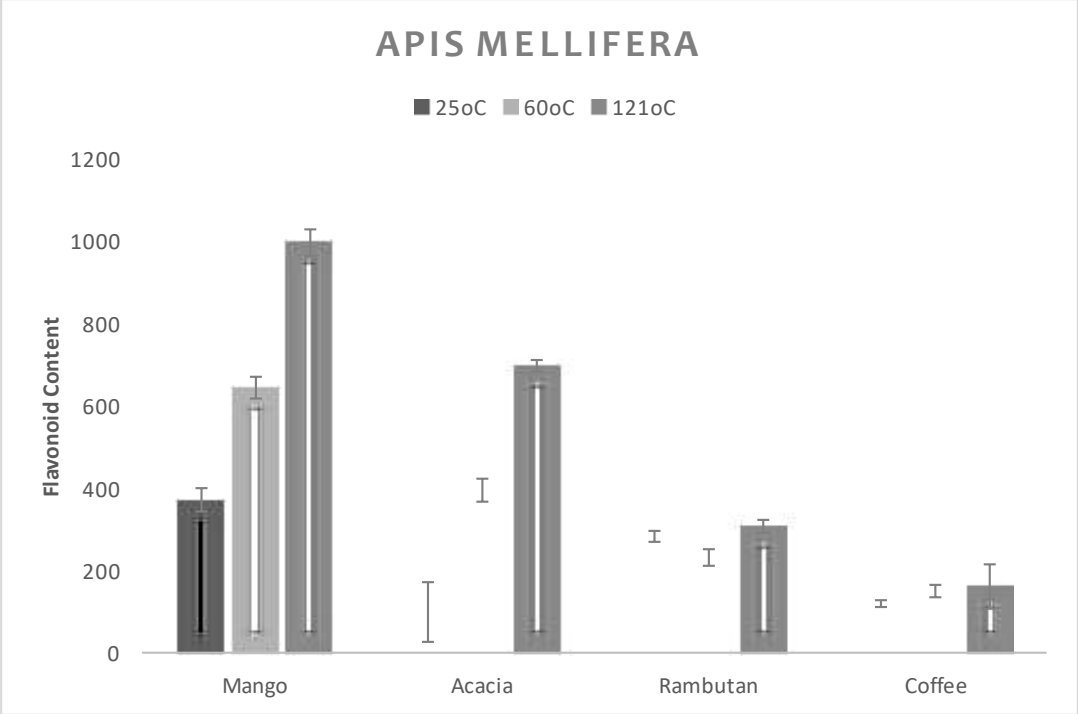

Fig. 2: Total flavonoid content of honey due to heating from Apis mellifera honey

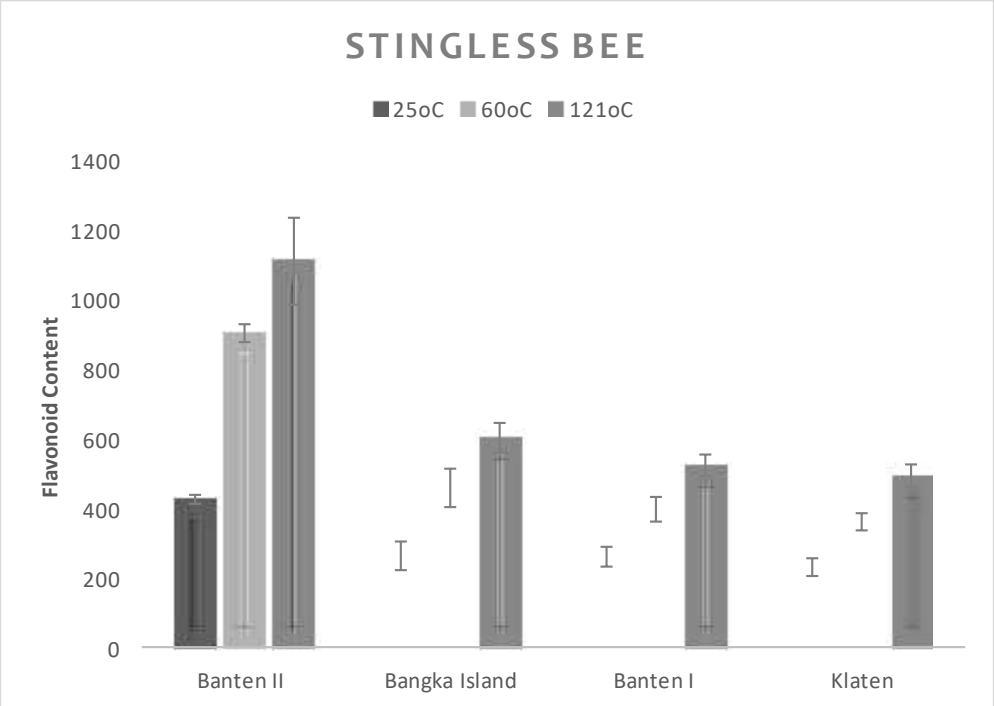

Fig. 3: Total Flavonoid content of honey due to heating from Stingles bee honey