Honey is rich in phenolic and flavonoid compounds that have the potential as antioxidants. The heating treatment of honey is also known to affect the honey compound. This study aimed to determine the best Indonesian *Apis dorsata* honey from various locations based on the level of total phenolics compounds, flavonoids, and antioxidant capacity due to variations in heating temperature. The procedures carried out in this study included: heating the sample with variations (room temperature, 65 °C, and 121 °C), measuring total phenolic content using the Folin-Ciocalteau method, measuring total flavonoid content using the AlCl₃ colorimetric method, and testing antioxidant activity using the DPPH method. Each honey's total phenolic, flavonoid, and antioxidant activity are affected by its origin. The impact of heating at 65 °C and 121 °C resulted in various changes between honey samples (p<0.05). The best *A. dorsata* honey overall was found in Flores honey after heating at 121 °C heating treatment with total phenolic content of 4.96 mgGAE/mL, total flavonoids of 1.173 mg QE/mL, and IC50 5.76 g/L.
1. INTRODUCTION

Free radicals are molecules that are highly reactive and unstable in the body. This is because free radicals contain unpaired electrons in their furthest layer. These highly reactive electrons find electron pairs by reacting to their surroundings to stabilize themselves. This reaction, which tends to attract electrons and transform a molecule into a new free radical in a chain reaction until a stable condition is reached, can occur simultaneously or continuously to avoid degenerative. This chain reaction must be stopped soon to prevent degenerative diseases like cancer, heart failure, and cataracts. Antioxidants are the only thing that can stop the process of free radical stabilization. Compounds known as antioxidants can combat free radicals by donating electrons to them, causing them to become stable and preventing free radical chain reactions. Antioxidants can come from food, outside the body, or the body itself. Vitamin C, vitamin E, vitamin A, organosulfur, -tocopherol, flavonoids, thymoquinone, statins, niacin, and phycocyanin are just a few of the local natural ingredients that contain antioxidants and various active substances (Werdhasari, 2014). Honey is one natural ingredient that can be an antioxidant source (Sumarlin et al., 2018).

Honey is a naturally occurring component with a thick consistency and a sweet flavor. Honey is defined as a natural liquid produced by bees (Apis sp.) from plant parts (extra floral) or flower extracts (floral nectar) that generally has a sweet taste, according to the Badan Standardisasi Nasional (2013). In Indonesia, there are numerous varieties of honey, including monoflora honey, multiflora honey, and kelulut honey. Forest bee honey, made by Apis dorsata bees, is one of Indonesia’s most widely produced honey. The community values honey as a commodity due to its numerous advantages. Honey’s primary nutrients come from various carbohydrate compounds like fructose, glucose, sucrose, and dextrins. Honey has antimicrobial, antiviral, antiparasitic, anti-inflammatory, anti-mutagenic, immunosuppressive, and anticancer properties (Gul & Pehlivan, 2018). It has likewise been demonstrated to be wealthy in phenolic and flavonoid contents, which are, for the most, which are mostly compounds (Olas, 2020).

The quality of honey differs incredibly depending upon many variables, including the wellspring of the plant, environment, ecological circumstances, and the types of honey bees. The quality of the honey bees can also be affected by how it is treated. In the honey industry, heating honey is one of the most common treatments. According to Singh & Singh (2018), heat treatment is typically used to reduce viscosity, remove microorganisms, reduce water content, and prevent crystallization and fermentation. According to Molaveisi et al. (2019), heating can alter honey’s physicochemical and antioxidant properties. From a nutritional point of view, changes in the antioxidant capacity of honey are significant and may alter honey’s health benefits. However, there still needs to be more information regarding the warming effects of A. dorsata honey in Indonesia. This study means determining the substance of total phenolic, flavonoid, and antioxidant activity of A. dorsata honey from various locations due to variations in heating temperature.

2. METHODS

2.1. Materials

This study used eight different varieties of honey samples: Gayo Lues, Siak, Bengkalis, Palembang, Garut, Pandeglang, Kapuas Hulu, and Flores. The materials used in this study were absolute methanol, distilled water, aquabides, Folin-Ciocalteau reagent, gallic acid, Na₂CO₃ 10%, AlCl₃ 10%, CH₃COOK 1 M, quercetin, DPPH, vitamin C, absolute methanol, TPTZ 10 mM, FeCl₃ 20 mM, 40 mM HCl, Trolox, and acetate buffer 0.3 M at pH 3.6. The tools used in this study were glassware, analytical balance, water bath (Benchmark), autoclave.
TOTAL PHENOLICS, FLAVONOIDS, AND ANTIOXIDANT ACTIVITIES OF INDONESIAN *Apis dorsata* HONEY DUE TO HEATING

Hasan et al.

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2.2. Sample Preparation

Honey samples were obtained from the beekeepers in August 2021, living across different locations throughout Indonesia, and collected by CV Madu Apiari Mutiara. After harvest, honey was kept at room temperature in jerry cans until the analysis. Before testing, samples were heated for five minutes at 65 °C using a water bath and 121 °C using an autoclave with a pressure of 1 atm. Sample tubes of each temperature were cooled before analysis. Then the honey was put into a smaller container according to the group. Then the honey can stand for a while until the temperature decreases. Unheated honey was used as a control.

2.3. Determination of Total Phenolic Content

The Folin-Ciocalteau method was used to determine the total phenolic content, and gallic acid was used as a calibration standard for spectrophotometric measurements. A total of 0.1 mL of honey was pipetted, and then 7.9 mL of distilled water and 0.5 mL of Folin-Ciocalteau reagent were added. After allowing the sample to stand for eight minutes, 1.5 mL of 10% Na2CO3 was added to the mixture. The solution was left to stand for 60 minutes at room temperature in the dark. Then the absorbance of the solution was estimated using a UV-Vis spectrophotometer at a wavelength of 725 nm. The test was carried out with three repetitions. Standard curves were calculated using gallic acid (20-100 g/mL) (Moniruzzaman et al., 2014). The total phenolic content is measured in mg gallic acid equivalent/mL honey, which is calculated using the formula:

\[
\text{Total phenolic (mg GAE/mL) } = \frac{\text{Total phenolic from curve (mg GAE L}^{-1})}{\text{Sample concentration (mL L}^{-1})} \times FP
\]

2.4. Determination of Total Flavonoid Content

Total flavonoids in honey samples were tested using the colorimetric method. A total of 1.5 mL of ethanol and 2.8 mL of distilled water were combined with 0.5 mL of the sample. After that, 0.1 mL of 10% AlCl3 was added. The solution was then added to 1 M CH3COOK in 0.1 mL. The solution was left to incubate for 30 minutes. UV-Vis spectrophotometer was used to measure the absorbance at 417 nm. The test was carried out with three repetitions. Calibration curves were constructed using standard quercetin solutions (20-100 g/mL) (Moniruzzaman et al., 2014). The result of total flavonoid content is measured in mg of quercetin equivalent (QE) per mL of honey, which is calculated using the formula:

\[
\text{Total flavonoid (mg QE/mL) } = \frac{\text{Total flavonoid from curve (mg QE L}^{-1})}{\text{Sample concentration (mL L}^{-1})} \times FP
\]

2.5. DPPH Antioxidant Activity Test

100 L of each honey sample (4, 6, 8, 10, 15, 20 g/L) added 100 L of dissolved DPPH reagent in methanol (0.125 mM). The mixture was kept in the dark for 20 minutes. A spectrophotometer was used to measure the solution’s absorbance at 517 nm. Three times were used for each measurement. The ability to reduce free radicals by 50% (IC50) indicates antioxidant activity. The IC50 value was obtained from the equation of the percent inhibition curve (Gul & Pehlivan, 2018).

\[
%\text{inhibition} = \left(\frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}}\right) \times 100\%
\]
2.6. Data Analysis

The results were presented as mean and standard deviation (SD) after each test was carried out in triplicate. Tremendous contrasts addressed by letters were gotten by one-way analysis of variance (ANOVA) trailed by Duncan’s subsequent test (P<0.05) on SAS programming variant 9.0. Combining honey types based on their content was carried out using principal component analysis (PCA) on Minitab software version 16.

3. RESULTS AND DISCUSSION

3.1. Results

The total phenolic content of *A. dorsata* honey was measured using the Folin Ciocalteu method. Gallic acid was used as a standard solution. The highest total phenolic content was found in Flores honey after 121 °C heating treatment of 4.96 mgGAE/mL and the lowest in Palembang honey heating at 65 °C, which was 0.35 mgGAE/mL (Figure 1). Based on the overall results showed that honey decreased in total phenolic content after heating treatment at 65 °C. However, the total phenolic content of honey increased after 121 °C heating treatment. Siak and Flores flavonoid data are secondary data from Hasan et al. (2021).

The total flavonoid of *A. dorsata* honey was measured using the AlCl$_3$ method. Quercetin was used as a standard solution. The highest total flavonoid content was found in Flores honey after 121 °C heating treatment (1.173 mgQE/mL), and the lowest was in Palembang honey after heating at 121 °C (0.095 mgQE/mL) (Figure 2). Based on the overall results, it showed that honey had an increase in total flavonoid levels after heating treatment at 65 °C and 121 °C, except for total flavonoid levels in Pandeglang honey decreased after 65 °C heating treatment and Gayo Lues honey and Palembang honey decreased after heating treatment at 65 °C and 121 °C.

![Figure 1 Total Phenolic Content of A. dorsata Honey Samples in Various Heating Treatments](image)

Note: The same letters on the diagram show that the results are not significantly different at the level of $\alpha = 0.05$ with Duncan.
Figure 2 Total Flavonoid Content of *A. dorsata* Honey Samples in Various Heating Treatments

Note: The same letters on the diagram show that the results are not significantly different at the level of $\alpha = 0.05$ with Duncan.

The antioxidant activity of honey was determined using the DPPH method with an IC$_{50}$ value. The IC$_{50}$ values obtained by each *A. dorsata* honey are shown in Figure 5. The smallest average IC$_{50}$ value at room temperature was found in Flores honey (12.49 g/L) and the highest in Pandeglang honey (79.21 g/L).

Figure 3 Inhibitory Concentration of 50 (IC$_{50}$) in *A. dorsata* Honey

Note: The same letters on the diagram show that the results are not significantly different at the level of $\alpha = 0.05$ with Duncan.

Honey clusterization from various locations based on total phenolic content, total flavonoid content, and IC$_{50}$ can be seen in Figure 4. PCA results are shown on a two-dimensional plot using the first two essential components (Figure 4), which together account
for PC1 (79%) and PC2 (16%) of the total 95% variation. The analysis demonstrates that forest honey can be grouped into four groups, the first group consists of Pandeglang honey, Garut honey, Palembang honey, and Kapuas Hulu honey; the second group is Siak honey and Bengkalis honey, then Gayo Lues honey, and Flores honey. Each honey from the same group is predicted to have the same environmental conditions. The climatic conditions of each honey location can be seen in Table 1.

![Score Plot](image)

**Figure 4** Plots of PCA Scores of PC1 and PC2 Forest Honey Samples

<table>
<thead>
<tr>
<th>Lokasi</th>
<th>Temperature average (°C)</th>
<th>Relative humidity average (%)</th>
<th>Rain rate average (mm)</th>
<th>Sunshine average (hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gayo Lues</td>
<td>26.46</td>
<td>89.56</td>
<td>26.08</td>
<td>4.59</td>
</tr>
<tr>
<td>Bengkalis</td>
<td>27.74</td>
<td>84.65</td>
<td>5.36</td>
<td>5.40</td>
</tr>
<tr>
<td>Siak</td>
<td>27.39</td>
<td>76.06</td>
<td>3.22</td>
<td>5.82</td>
</tr>
<tr>
<td>Palembang</td>
<td>27.69</td>
<td>81.15</td>
<td>9.59</td>
<td>5.33</td>
</tr>
<tr>
<td>Pandeglang</td>
<td>27.22</td>
<td>78.68</td>
<td>1.56</td>
<td>6.16</td>
</tr>
<tr>
<td>Garut</td>
<td>23.17</td>
<td>72.87</td>
<td>1.84</td>
<td>7.15</td>
</tr>
<tr>
<td>Kapuas Hulu</td>
<td>27.15</td>
<td>81.46</td>
<td>10.08</td>
<td>5.74</td>
</tr>
<tr>
<td>Flores</td>
<td>27.96</td>
<td>67.54</td>
<td>0</td>
<td>7.08</td>
</tr>
</tbody>
</table>

### 3.2. Discussion

#### 3.2.1. Total Phenolic Content

Flores honey had the highest total phenolic content after being heated at 121 °C (4.96 mgGAE/mL), and the lowest was found in Palembang honey after heating at 65 °C (0.35 mgGAE/mL) (Figure 1). Significant differences existed between the total phenolic content and the origin of the honey (one-way ANOVA; p < 0.05). Honey’s floral origin might cause the differences. The variety of flower nectars from each region was probably to blame for the
significant difference in the total phenolic contents from various geographical regions (Sánchez et al., 2021).

Heat treatment at 65 °C significantly reduced the total phenolic content in Gayo Lues and Flores honey. These results are similar to previous studies; the impact of heating at 65 °C diminished the total phenolic content (Zarei et al., 2019) because heat-sensitive phenolic compounds degrade. On the other hand, Bengkalis honey, Siak honey, Palembang honey, Pandeglang honey, Garut honey, and Kapuas Hulu honey showed no significant differences when heated at 65 °C. The interaction of total phenolic contents after heat treatment varied among the types of honey depending on where the honey originated (Escriche et al., 2014).

Heat treatment at 121 °C significantly increased the total phenolic content in 4 kinds of honey. According to Aydogan-Coskun et al. (2019), some phenolic compounds can rise because supramolecular structures with phenolic groups are broken down. Applying pressure when heating to 121 °C in an autoclave can also enhance the hydrogen bonding of the hydroxyl groups in phenolic compounds so that they become more stable (Fauzi et al., 2013).

3.2.2. Total Flavonoid Content

The highest flavonoid content was found in Flores honey after heating at 121 °C (1.173 mgQE/mL) and the lowest in Palembang honey after heating at 121 °C (0.095 mgQE/mL) (Figure 2). Siak and Flores flavonoid data are secondary data from Hasan et al. (2021). Overall results show that the total flavonoid content of honey increased after heating at 65 °C and 121 °C, except for the Pandeglang honey decreased after heating at 65 °C, Leuser honey and Palembang honey lowered after heating at 65 °C and 121 °C.

Total flavonoids reacted differently after heating when compared to total phenolics. Differences in the structure of these compounds can affect their stability in heat processing (Chaaban et al., 2017). The increase in honey’s total flavonoids after heating may be due to pollen disintegration and is associated with a rise in the extraction ability of several components like flavonoids (Chaikham et al., 2016).

3.2.3. DPPH Antioxidant Activity

Honey is known to have antioxidant properties (Cahyaningrum, 2019). Honey contains bioactive substances like vitamins, flavonoids, anthocyanins, and phenolic acids responsible for antioxidant activity (Chaikham et al., 2016). Honey’s capacity to neutralize free radicals was investigated in this study at various concentrations. The amount of honey needed to reduce the amount of DPPH by 50% (IC₅₀) has been calculated, and the results are shown in Figure 3. The lower the IC₅₀ of a sample, the higher it is antioxidant activity (Abdullah et al., 2014). Flores honey showed significantly higher DPPH-scavenging activity than other kinds of honey, which had an IC₅₀ of 12.49 g/L. This is related to the polyphenol contents where Flores honey has the highest total phenolic and flavonoid content.

The outcomes revealed a rise in IC₅₀ in the heating honey compared to the control indicating that the heating process could release antioxidant compounds in honey. During heating, the interaction between sugar compounds and amino acids causes a series of changes in honey’s composition. In the final stage, the Maillard reaction, also known as non-enzymatic browning, breaks down Amadori products and produces aldehydes, pyrazines, pyrroles, and furans (Elamine et al., 2020). Melanoidin molecules are produced when active molecules combine with amino acids to form a condensation (Elamine et al., 2020). As a result of the Maillard reaction product, the heating treatment increases honey’s antioxidant capacity (Escriche et al., 2014).

Heating honey also causes a caramelization reaction. During high-temperature treatment, caramelization is a non-enzymatic browning reaction that gives sugars a caramel-like flavor. The Maillard reaction can occur at more moderate temperatures, but the sugars caramelize above 120 °C (Kocadağlı & Gökmen, 2018). During caramelization, a few
mixtures come from sugar debasement. These compounds influence Caramel’s flavor, aroma, color, and antioxidants. Caramel’s aldehydes and furans are antioxidants (Rahardjo et al., 2020). The results showed that heating at 121 °C resulted in higher antioxidant changes than heating at 65 °C due to the product of the Maillard reaction and caramelization at 121 °C heating.

3.2.4. Honey Clusterization

PCA results are shown that forest honey can be grouped into four groups, the first group consists of Pandeglang honey, Garut honey, Palembang honey, and Kapuas Hulu honey; the second group is Siak honey and Bengkalis honey, then Gayo Lues honey, and Flores honey. The grouping of A. dorsata honey based on total phenolic content, total flavonoid, and antioxidant activity can be influenced by plant diversity from each location and environmental conditions such as weather, temperature, and rainfall (Mutiah et al., 2018). Each honey from the same group is predicted to have the same environmental conditions. The climatic conditions of each honey location can be seen in Table 1. The climatic conditions in Table 2 are conditions for one month before honey harvesting. The results of PCA analysis on Flores honey showed a different point from other groups. According to Meteorology Climatology and Geophysics Council (2021), the East Nusa Tenggara region had a long day without rain in July 2021. Stress conditions such as high temperatures and low rainfall can affect the production of secondary metabolites produced by plants as an adaptation response to the environment (Utomo et al., 2020).

4. CONCLUSION

Each honey’s total phenolic, flavonoid, and antioxidant activity are affected by its origin. The impact of heating at 65 °C and 121 °C resulted in various changes between honey samples (p<0.05). The best A. dorsata honey overall was found in Flores honey after heating at 121 °C heating treatment with total phenolic content of 4.96 mgGAE/mL, total flavonoids of 1.173 mg QE/mL, and IC50 5.76 g/L.

REFERENCES


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