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Isolation and Molecular Weight Characterization of *Tetragonula laeviceps* Honey Protein

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Abstract

The concentration of protein in honey is lower than polysaccharide. However, recently the honey’s protein is intensively studied and it showed that protein also have several biological activities such as antibacterial activity. The purpose of this research is to isolate and characterize *Tetragonula laeviceps* honey protein by determining the molecular weight. Honey protein of *Trigona laeviceps* was isolated using ultra-filtration with the membrane’s size of 30 kDa, then concentrated using tube membrane size 10 kDa. Molecular weight was analyzed by SDS PAGE. From the analysis, there was major protein band in honey produced by *Tetragonula laeviceps* identified. The produced molecular weight of major protein bands were about 87 and 65 kDa. Determining of the molecular weight of this protein could be used to detect the originality of *Tetragonula laeviceps* honey from Indonesia.

1. Introduction

Honey is a natural liquid, generally have sweet taste and produced by bees from flower’s nectar of the plant (nectar floral) or other parts of plants (extra nectar floral) or excretion of insects [1]. Honey is a natural sweetener that has been used as a food source since 6000 years ago [2].

Honey has chemical constituents such as sugars [3], hydroxymethylfurfural (HMF), by-products of invert sugars [4], flavonoids [5], as well as minerals and proteins [6]. These constituents are affected by various floral and regional sources as well as the storage period of honey, which can affect its quality value [7].

Pure honey is taken directly from nature has a relatively high price for the difficult process. This triggers the addition of water, sugar and other substances to increase the volume of honey produced. The addition of other substances (honey adulteration) in the honey spoil its purity.

Counterfeiting honey like adding substances such as corn syrup, maple syrup, sugar cane, sugar beet, molasses, invert sugar and so on, becomes a problem in Indonesia. The authenticity of honey is difficult to detect just by evaluating the external appearance of honey. This is also a widespread problem experienced by consumers. Some types of honey from a particular types of nectar have a higher price. Honey sellers can deceive consumers by...
manipulate the flavor, color and aroma of honey to match the honey from the specific types of flora.

Manipulation of honey volume by adding water is also done by the seller. In fact, high water content can cause fermentation in honey. Fermented honey will cause discomfort in the honey and increased the number of dead yeast in honey. Fermented honey also contains glycerol, butanediol [8] and alcohol [8]. However, only honey that has a moisture content above 25% has risk of fermentation [8].

Proteins are the trace elements in honey and amounts to between from 0.1 to 0.4% [8]. In a study conducted the major protein in honey have different molecular weights, depending on the species of bees producing honey [7].

There is another approach based on analysis of the composition or the origin of proteins in honey. At least nineteen protein bands were detected by silver-staining SDS-PAGE in honeys with different plant origin [9]. *Trigona laeviceps* honey from Thailand also had been investigated by obtaining the type of major protein, an antimicrobial activity on various pathogens [10].

From the above problems, therefore it is necessary to characterize protein from the original honey of Indonesia so that it becomes a standard of authenticity. The aim of this research is to isolate and characterize *Tetragonula laeviceps* honey protein by determining the molecular weight.

2. Methods

**Sample Collection.** Honey of *Trigona laeviceps* was taken from Universitas Indonesia’s forest, Depok, West Java, Indonesia. Sample was stored at 25 °C until used.

**Pre Isolation.** In this study, there was pre-isolation before doing isolation. Pre-isolation used dialysis method to remove sugars and other substances. Dialysis was done by adding honey’s protein sample with 3 liters of distilled water for 24 h. After dialysis, sample was centrifuged with 5000 rpm for 30 minutes at 4 °C.

**Isolation.** Protein isolation was done using ultra filtration with N₂ pressure using size membrane 30 kDa. N₂ pressure was given from 3 MPa to 0.18 MPa. After that, sample was concentrated using ultrafiltration Millipore TM to get more concentrated protein from honey with membrane size of 10 kDa.

**SDS Page Analysis.** SDS PAGE is based on a system of Laemmli (1970). SDS PAGE Laemmli system was done using four components: main buffer electrophoresis, the sample solution, running gel and stacking gel.

This study used running gel 7.5% (w/v) and the composition are: 3.7 mL distilled water, 2 mL of 30% acrylamide, 2 mL of 1.5 M Tris-HCl with pH 8.8, 0.08 mL of 10% SDS, 0.054 mL of 10% APS, and 0.008 mL of TEMED.

In addition, this experiment used stacking gel 4.0% (w/v) with the composition: 1.44 mL of distilled water, 0.4 mL of 30% acrylamide, 0.625 mL of 1.5 M Tris-HCl with pH 8.8, 0.025 mL of 10% SDS, 0.0166 mL of 10% APS, and 0.005 mL of TEMED.

Running gel 7.5% (w/v) was inserted into the printer apparatus slab gel from Bio Rad Mini Protein II at the bottom until the solution reached 2 cm of the top of the glass gel. Running gel will then harden and afterwards included stacking gel 4.0% (w/v) above separating gel, and the stacking gel which is still liquid inserted comb to make a well. These wells used to insert the protein samples to be characterized in the gel. The gel was prepared fed into an electrophoresis.

Sample and the marker protein incorporated into wells gel on a tool electrophoresis with volume 25 µL. Electrophoresis was performed at the starting 30 mA to rate ribbon line with the lower limit of the gel. Then, Gel was soaked in distilled water and put in the microwave for 2 minutes, this step was repeated until three times. Then, Gel stained with dye solution (staining) and inserted into in microwave for 2 minutes.

3. Results and Discussion

**Protein Isolation.** To isolate protein from *Trigona laeviceps* honey, pre isolation is required as a pre-treatment prior to isolation and analysis done. Pre isolation is useful to ease the isolation process of proteins from *Trigona laeviceps* honey. In this study, dialysis was done as pre isolation of protein. Dialysis was done for 24 hours at 24 °C using membrane size: 6-8 kDa.

Protein can be separated from small molecules by dialysis through a semipermeable membrane, such as a cellulosic membrane with pores. Molecules having dimensions significantly greater than the pore diameter are retained inside the dialysis bag, whereas smaller molecules and ions traverse the pores of such a membrane and emerge in the dialysate outside the bag. This technique is useful to remove salt or other small molecules, but it will not effectively distinguish proteins [11]. After that, sample was centrifuged. Other substances can be removed by centrifugation so that the proteins and other soluble compounds remained in the supernatant.

The next isolation was done using filtration with N₂ pressure with Millipore stirred cell with size membrane 30 kDa. Volume of sample used was 170 mL. Honey sample volume decreased until 8 ml. After that, sample can be checked with Bradford solution to check the presence of protein qualitatively. To get more concentrated protein,
sample was concentrated using Millipore TM tube with size membrane 10 kDa for 10 times. After that, sample can be analysed with SDS Page to determine the molecular weight of *Tetragonula laeviceps* honey protein.

**Determining Molecular Weight of *Tetragonula laeviceps* honey protein from SDS Page Analysis.** Many variations of honey include it is properties because of different locations, seasons and bee species [10]. Therefore, *Trigona laeviceps* honey from Indonesia was selected and evaluated to know the characteristic especially molecular weight of this honey protein to detect the originality of *Trigona laeviceps* honey from Indonesia.

After isolation and concentration of honey protein, sample was analysed with SDS Page to know the molecular weight of *Tetragonula laeviceps* honey protein. With SDS Page, could be seen many proteins in *Tetragonula laeviceps* honey.

In SDS Page analysis used 7.5% running gel because from trials used 15% but the protein band result was not clear. Before running on SDS page, sample was prepared by mixing the sample with sample buffer. 200 µL of honey protein sample added with 100 µL of 4 times of sample buffer. Volume sample which was given for each comb for same sample of *Tetragonula laeviceps* honey protein was 25 µL.

Honey protein was resolved by one dimensional SDS page instrument. After SDS page, then gel of sample was stained by commasie blue solution and after that destaining the gel to see the bands of sample of honey protein. From the results, was known that there were 5 bands of protein detected on the gel. However, only two major protein could be seen. The bands can be seen in Figure 1.

From Figure 1, it was known that there were 5 bands, but there was one major band from *Trigona laeviceps* honey protein. The result showed only two major band which were detected the molecular weight. Molecular weight was determined by calculating the size based on SDS Page.

Calculating size of molecular weight based on SDS Page was known by determining Rf value = distance migrated/gel length for the standard and the experiment’s result. After that, log (mol.wt) against Rf was plotted for standard and extrapolated size of the experiment’s result. From the calculation, it was known that Table 1.

From Figure 2, molecular weight of *Trigona laeviceps* honey protein sample could be calculated. The main protein known that Rf of sample is 0.47 by input to the equation, the log is 4.93 Dalton. From extrapolating, size of molecular weight of *Tetragonula laeviceps* major honey protein was 87 kDa. The other protein have Rf 4.81 of sample and the molecular weight is about 65 kDa.

Each protein from different bee species has different molecular weight. Differences in molecular weight of these major proteins in Korean honey were noted *Apis cerana* of 56 kDa and *A. mellifera* of 59 kDa [12]. It also shows the difference of molecular weight resulting from *Tetragonula laeviceps* major honey from Indonesia. From the difference of the result, we can distinguish character of each honey bee species.

### Table 1. Rf Value of Protein Marker

<table>
<thead>
<tr>
<th>size (kDa)</th>
<th>Log</th>
<th>Cm</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>5.301</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>116</td>
<td>5.065</td>
<td>1.8</td>
<td>0.3</td>
</tr>
<tr>
<td>97</td>
<td>4.988</td>
<td>2.4</td>
<td>0.4</td>
</tr>
<tr>
<td>66</td>
<td>4.820</td>
<td>3.2</td>
<td>0.53</td>
</tr>
<tr>
<td>45</td>
<td>4.653</td>
<td>4.85</td>
<td>0.81</td>
</tr>
</tbody>
</table>

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*Figure 1. Bands of *Trigona laeviceps* Honey Protein. Lane 1: Broad Range Protein weight Markers with Sizes in kDa shown in the Left. Lane 2-9: all of *Trigona laeviceps* Honey Protein. The Red Circles are main Protein Bands*
To know more specific molecular weight and specific kind of *Trigone laeviceps* honey protein, it should be done with more isolation/purification of protein then analysed with mass spectrometry analysis. Therefore, this sample result should be continued to know its sequence of amino acid.

4. Conclusions

*Trigona laeviceps* honey protein was isolated and characterized. From isolation, it was known the molecular weight of main *Trigona laeviceps* honey proteins are 87 and 65 kDa. Determining the molecular weight of this protein also could be used to detect the originality of *Tetragonula laeviceps* honey.

References