Analysis of Total Protein and Non Protein Nitrogen in Pakkat (Calamus caesius Blume.) as a Traditional Food of Mandailing Natal by using Kjeldahl Method

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Abstract: Pakkat is a traditional food in Mandailing Natalis taken from the inside of the young rattan and the most commonly consumed by people is grilled pakkat. Pakkat can be used as vegetables and believed to cure many diseases that need to be researched. The content of nutrients in it is expected to take it become one of the functional food. The aim of this study was to determine the concentration of total protein and non protein nitrogen (NPN) in pakkat and their changes in fresh, grilled and boiled pakkat.

The samples used in this study is young rattan from the forest of Lumban Pasir village, Mandailing Natal, North Sumatera. Total protein and NPN determination is done by using Kjeldahl method which is a simple method for total nitrogen determination in protein and other nitrogenous compounds.

The results show that the total protein contents in fresh, grilled and boiled pakkat are 6.00 g/100g, 4.84 g/100g and 3.07 g/100g respectively. NPN contents in fresh, grilled, and boiled are 0.600 g/100g, 0.488 g/100g and 0.315 g/100g respectively. Pure protein contents in fresh, grilled, and boiled are 2.25 g/100g, 1.79 g/100g and 1.10 g/100g respectively. The results show that total protein and NPN contents in fresh pakkat is higher than in grilled and boiled pakkat.

Keywords: fresh pakkat, grilled pakkat, boiled pakkat, protein, non protein nitrogen, kjeldahl.

Introduction

The inside of the young rattan that can be eaten in Mandailing Natal area called the "pangkat", while in the Medan, North Sumatera, Indonesia called "pakkat". Pakkat is a unique food when fasting the citizens in Mandailing Natal and Medan and the most commonly consumed by people is grilled pakkat. Young rattan burned on a stove about 15 minutes and after certain rattan ripe, peeled and taken part in the white cane. Then the meat rattan cut, it's not too bitter, not smell in the mouth and has a texture that is soft and easy to chew. Besides, it can also be consumed fresh or by boiling. The boiling process is beneficial to remove the taste bitter. Pakkat is very interesting because it is believed to cure various diseases such as diabetes and malaria, also have efficacy as a whet your appetite when fasting. So that pakkat is expected to be used as functional food. Functional foods as any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains1, 2.

Rattan, a spiny climbing or trailing palm with some 600 species, is strictly an old world plant. Indonesia, where half of the known species grow, is the world's largest producer of rattan raw materials. Cane, the stem of rattan minus the sheaths, is the most valuable part of the plant. Rattan is a collective term commonly used for spiny palms of the family Arecaceae. Both fruits and shoots of rattan are edible, and the latter contain...
high amounts of protein, carbohydrates, amino acids, vitamins, and other nutrients. Rattan roots, fruits and leaves are used in traditional medicine. Proteins are polymers of amino acids that are covalently joined by a substituted amide linkage named a peptide bond. There are 20 different amino acids that make up food proteins.

Food protein are essential source of amino acid in the diet which are necessary for normal growth and maintenance of the body. Food proteins have two major functions are providing energy and essential nutrients to humans, and imparting the physicochemical characteristics that give rise to unique quality and sensory attributes of food, such as texture. Although proteins have numerous biological functions in vivo as enzymes, hormones, and antibodies or defense mechanism of the body. The Kjeldahl and visible spectrophotometry method commonly used for analysis total protein and non protein nitrogen.

The aim of this study was to determine the change in total protein content in fresh, grilled and boiled pakkat. Additionally done also test the determination of organoleptic and water content observations against pakkat. Total protein and non protein nitrogen determination is done by Kjeldahl method.

**Experimental**

**Apparatus**

Apparatus used in this study were analytical balance(Mettler), Kjeldahl flask (FOSS), destruction tool (scrubber and heating), distillation apparatus(UDK 130 A), oven, (Pyrex), hot plate(Nouva), magnetic stirrer(Pyrex), burette(Pyrex), and laboratory glassware.

**Samples**

Pakkat used in this study were derived from young rattan planted of forest in Lumban Pasir village, Mandailing Natal. Length of young rattan taken about 70 cm and used as much as 10 rods. The identification of plants done in the Herbarium Medanense, Herbarium Laboratory Faculty of Mathematics and Natural Sciences, University of North Sumatera.

**Chemical materials**

Chemical materials used in this study were 98% concentrated sulfuric acid, selenium dioxide, cupric sulfate, potassium sulfate, 40% sodium hydroxide, 4% boric acid, methyl red, methylene blue, 37% concentrated hydrochloric acid, 10% trichloroacetic acid, sodium tetraborate and distilled water.

**Samples Preparation**

Fresh pakkat: young rattan has been cleared taken part in white and then blended, and weighed 1g fresh pakkat to be used for total protein and NPN determination.

Grilled pakkat: young rattan has green outer skin is grilled using firewood for ±15 minutes until the outer skin coloured black, cooling, take part in white young rattan and blended, weighed 1g grilled pakkat to be used for total protein and NPN determination.

Boiled pakkat: young rattan has been cleared taken part in white, boiling with boiled water at 100°C with a ratio boiled pakkatas much as 100 g and the water used 500ml for ±15 minutes until pure white with a boiling. Cooling and blended and then weighed 1g boiled pakkat to be used for total protein and NPN determination.

**Standardization of 0.01N Hydrochloric Acid Solution**

0.01g of sodium tetraborate (Na₂B₄O₇·H₂O) was weighed then placed into 100 ml erlenmeyer and 10ml distilled water. After soluble, added 2drops of indicator methyl red and titrated with 0.01N hydrochloric acid solution to best and ardized until a pale yellow color. Forward titration, titration was done until the solution color is rose-pink.
Determination of Water Content

Determination of water content was done by Gravimetry method. Fresh pakkat has been blended, then weighed quickly as much as 2g into a porcelain crucible of known weight and was dried for 30 minutes at 105ºC. Levelled by shaking slowly, inserted into the oven at 105ºC for 3 hours and cooling in desiccator and weighed. Repeat the heating, cooling and weighing until a constant weight is obtained. Weighing the sample in water content can be expressed by wet basis or dry basis.

\[
\text{Water Content (\%) = } \frac{a}{b} \times 100\%
\]

Where: a) weight of samples before be dried, b) loss of weight after be dried.

Determination of N-Total and Total Protein Content

1 g sample was weighed and placed into Kjeldahl flask, then 1 g of catalyst selenium and 25 ml of concentrated sulfuric acid were added. Digestion was done for about 30 minutes until the color of the digest is clear and cooled. After it was cooled, entering into a 100 ml of flask, matched up to the mark with distilled water. Pipette 25 ml of solution and add 50 ml of 40% sodium hydroxide was put into the distillation apparatus. 25 ml of 4% boric acid and 3 drops of indicator mengsel were added into the receiving flask. The distillate were titrated with 0.01 N hydrochloric acid solution until the destillate colour changes from emerald green to purple. Carry out a blank determination in the same way without the sample.

Determination of total protein content was conducted on a wet basis samples, then the total protein content of the dry basis samples mathematically derived by converting the total protein content in the wet basis samples be a total protein content in the dry basis samples.

N-total content was calculated using the following expression:

\[
\% \text{ N-total} = \frac{\text{Volume (ml) } HCl \text{ (sample-blank)}}{\text{weight of sample (g) x 1000}} \times N \text{ HCl} \times 14.007 \times \text{Dilution Factor} \times 100\%
\]

where, \(N \text{ HCl} = 0.0117 \text{ N}\)

Total protein content was calculated using the following expression:

\[
\text{Total Protein (\%) = } \% \text{ N-total} \times \text{Conversion Factor}
\]

where, conversion factor for pakkat = 6.25°.

Separation of Protein from Non Protein Nitrogen

Separation of protein from NPN was done by precipitating protein in the samples using 10% trichloroacetic acid. Samples was weighed and placed into 200 ml glass beaker. 50 ml of distilled water was added and allowed to stand for 30 minute. 10 ml of 10% trichloroacetic acid was added, allowed to stand for 30 min, and then filtered. The precipitate which contained true protein was washed twice with 90% trichloroacetic acid solution.

Determination of N-Protein and Pure Protein Content

Pure protein content was determined after separation process from NPN. The nitrogen content in protein precipitate obtained was determined by using Kjeldahl method, as was done for the determination of total protein. Protein precipitate was placed into Kjeldahl flask. 1 g of catalyst selenium and 25 ml of concentrated sulfuric acid were added. The next procedure is as same as the procedure for total protein determination.
N-protein content was calculated using the following expression:

\[
\% \text{N-protein} = \frac{\text{Volume (ml) HCl (sample - blank)}}{\text{weight of sample (g) \times 1006 \times N HCl \times 14.007 \times Dilution Factor}} \times 100\%
\]

where, \( N \text{HCl} = 0.0117 \text{ N} \)

Pure protein content was calculated using the following expression:

\[
\text{Pure Protein (\%)} = \% \text{N-total} \times \text{Conversion Factor}
\]

where, conversion factor for pakkat = 6.25\(^{\circ}\).

**Determination of Non Protein Nitrogen Content**

Non protein nitrogen content was calculated by subtracting N-protein from N-total\(^{11}\). Non protein nitrogen content in samples was calculated using the following expression:

\[
\% \text{NPN} = \% \text{N-total} - \% \text{N-protein}.
\]

Non protein nitrogen content expressed as percent of total nitrogen was calculated by using the following expression:

\[
\text{NPN content as percent of N-Total (\%)} = \frac{\% \text{NPN}}{\% \text{N-total}} \times 100\%
\]

**Data Analysis using Statistics**

Nitrogen and protein contents in each samples were analyzed using t-test standard deviation method. Standard deviation was calculated using the following expression:

\[
SD = \sqrt{\frac{\sum (X - \overline{X})^2}{n - 1}}
\]

Data was rejected if \( t \text{ value} \geq t\text{-table} \) at the confidence interval of 99\% (\( \alpha = 0.01 \)). \( t \)-value was calculated by using the following expression:

\[
t\text{value} = \left| \frac{X - \overline{X}}{SD \sqrt{n}} \right|
\]

where, \( SD = \text{Standard deviation} \)

\( X = \text{Protein content} \)

\( \overline{X} = \text{Mean of the protein content} \)

\( n = \text{number of determinations} \)

The actual protein content was calculated using the following expression:

\[
\mu = \overline{X} \pm t\text{-table} \times \frac{SD}{\sqrt{n}}
\]

where, \( \mu = \text{Actual protein content} \)

\( \overline{X} = \text{Mean of the protein content} \)

\( SD = \text{Standard deviation} \)

\( n = \text{number of determinations} \)^{12,13}.
Results and Discussion

Identification of Plant

Identification of plant result shows that samples used is pakkat (Calamus caesius Blume.) from family Arecaceae.

Organoleptic Samples

Organoleptic observations in pakkat can be seen in Table 1 below:

Table 1. Organoleptic observations in pakkat

<table>
<thead>
<tr>
<th>Description</th>
<th>Fresh Pakkat</th>
<th>Grilled Pakkat</th>
<th>Boiled Pakkat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter</td>
<td>1.2 cm</td>
<td>1.2 cm</td>
<td>1.2 cm</td>
</tr>
<tr>
<td>Outer skin</td>
<td>Green</td>
<td>Black</td>
<td>Green</td>
</tr>
<tr>
<td>Pakkat colour</td>
<td>White</td>
<td>White</td>
<td>Pure white color</td>
</tr>
<tr>
<td>Taste</td>
<td>Extremely bitter (++++)</td>
<td>Bitter (+++)</td>
<td>Bitter (+++)</td>
</tr>
<tr>
<td>Texture</td>
<td>Hard</td>
<td>Soft</td>
<td>Soft</td>
</tr>
</tbody>
</table>

Water content in the samples

Determination of water content in the samples was done by Gravimetry method. The water content in fresh, grilled and boiled pakkat obtained can be seen in Table 2 below:

Table 2. Results of Water Content in Fresh Pakkat, Grilled Pakkat and Boiled Pakkat

<table>
<thead>
<tr>
<th>Samples</th>
<th>Water Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Pakkat</td>
<td>88.68</td>
</tr>
<tr>
<td>Grilled Pakkat</td>
<td>88.25</td>
</tr>
<tr>
<td>Boiled Pakkat</td>
<td>90.66</td>
</tr>
</tbody>
</table>

Each value represents an average of six replications (n=6)

Based on the table above the water content in boiled pakkat (90.66%) is higher than the water content of fresh pakkat (88.68%) and grilled pakkat (88.25%). This is because the boiling process uses water so pakkat will absorb the water that causes the water level to be increased and the structure becomes softer. While grilled pakkat the water will evaporate during the combustion process that causes the water content of this grilled pakkat will be reduced.

Total Protein, Pure Protein and Non Protein Nitrogen Contents in Samples

Total protein content, pure protein and NPN content on boiled pakkat smaller than in fresh and grilled pakkat. This is likely due to the effect of treatment in the form of grilled and boiling. Heat treatment on food will increase the solubility of proteins, especially when grilled or boiled. Many agents that can cause changes in the nature of the protein for example heat, acids, bases and heavy metals.

When measured on samples of wet basis total protein content and pure protein on a fresh pakkat are 6.00 g/100g and 2.25 g/100g respectively. In these results are higher than the levels of total protein and pure protein on grilled pakkat 4.84 g/100g and 1.79 g/100g, and the boiled pakkat 3.07 g/100g and 1.10 g/100g. In dry basis samples, total protein content and pure protein on a fresh pakkat 53.03 g/100g and 19.91 g/100g. The higher than the total protein and pure protein content in grilled pakkat that is 41.25 g/100g and 15.26 g/100g and boiled pakkat 32.9 g/100g and 11.76 g/100g.
The results of N-total, N-protein, total protein, pure protein and non protein nitrogen determination in pakkat are shown in Table 3.

### Table 3. N-Total, N-Protein, Total Protein, Pure Protein and Non Protein Nitrogen Contents

<table>
<thead>
<tr>
<th>Samples</th>
<th>Content (g/100g)</th>
<th>N-Total</th>
<th>N-Protein</th>
<th>Total Protein</th>
<th>Pure Protein</th>
<th>NPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Pakkat</td>
<td></td>
<td>0.961±0.0025</td>
<td>0.361±0.0016</td>
<td>6.00±0.016a</td>
<td>53.03±1.1156b</td>
<td>2.25±0.0104a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19.91±0.4114b</td>
<td>0.600</td>
<td>62.43*</td>
</tr>
<tr>
<td>Grilled Pakkat</td>
<td></td>
<td>0.775±0.0031</td>
<td>0.287±0.0013</td>
<td>4.82±0.0232a</td>
<td>41.25±0.247b</td>
<td>1.79±0.0104a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15.26±0.2471b</td>
<td>0.488</td>
<td>62.97*</td>
</tr>
<tr>
<td>Boiled Pakkat</td>
<td></td>
<td>0.491±0.0025</td>
<td>0.176±0.0043</td>
<td>3.06±0.0194a</td>
<td>32.9±0.5095b</td>
<td>1.10±0.0265a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.76±0.2706b</td>
<td>0.315</td>
<td>64.15*</td>
</tr>
</tbody>
</table>

Each value represents an average of six replications (n=6)

a) content in wet basis, b) content in dry basis

*: content expressed as percent of total nitrogen in samples

Based on the Table 3, found that greater levels of NPN contained in fresh pakkat (0.600 g/100g) than Grilled pakkat (0.488 g/100g) and boiled pakkat (0.315 g/100g). Non Protein Nitrogen decreased levels can be caused by differences in the properties of the amino acids found in protein molecules. In general, the amino acid is soluble in water and will orient toward the surface of the molecule and try to interact with water. So that the levels of NPN in grilled and boiled pakkat will be reduced because of the evaporation of nitrogen that occur in the grilled and boiled process. Processing by heat is hypothesized to increase food digestibility due to breakdown of complex proteins. Protein also increased in all cooked sample. This decreased the water content, thereby causing dehydration-associated changes, such as an increased protein concentration.

When measured against N-total samples, the levels of the processing turns NPN considerable influence and the highest is in boiled pakkat NPN where the levels it reached 64.15% of the total content of nitrogen in the samples. Then NPN levels in grilled pakkat decreased (62.97%), and the lowest found in fresh pakkat (62.43%). The significance of nonprotein, organic, nitrogenous compounds in foods has been appreciated only in recent years. These compounds include amino acids, amines, amides, quaternary nitrogen compounds, purines, pyrimidines and N-nitrosamides. They contribute to nutritional value, flavor, color and other important food attributes.

### Conclusion

Total protein content were obtained on a samples of fresh, grilled and boiled pakkat are 6.00g/100g, 4.84g/100g and 3.07g/100 g respectively. NPN levels obtained in samples of fresh, grilled and boiled are 0.600g/100g, 0.488g/100g and 0.315g/100g respectively. The results obtained indicate that there is a difference between the levels of total protein and pure protein between fresh, grilled and boiled pakkat. Total protein, NPN, and the pure protein content on a fresh pakkat higher than grilled and boiled pakkat.

### References


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