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Analysis of Total Protein and Non Protein Nitrogen in Coconut Water and Meat (*Cocos Nucifera* L.) by using Kjeldahl Method

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Abstract : In Indonesia, coconut palm is one of the biggest contributors for the economy of the people and nation. As food, coconut water and coconut meat contain some nutrients such as carbohydrate, fat, and also protein. During maturation, changes in protein content of coconut water and coconut meat may happen. The aim of this study was to determine the concentration of total protein and non protein nitrogen (NPN) in coconut water and coconut meat, and their changes in young and mature coconuts.
The samples used were young and mature coconuts which were obtained from a villager's garden located at Desa Saintis, Kecamatan Percut Sei Tuan, Kabupaten Deli Serdang, Medan. Total protein and NPN determination were done for coconut water and coconut meat by using Kjeldahl method which is a simple method for total nitrogen determination in protein and other nitrogenous compounds.
The result shows that the total protein contents in dry samples are: young coconut water 2.19 g/100g; mature coconut water 1.13 g/100g; young coconut meat 4.14 g/100g; mature coconut meat 2.87 g/100g. Total protein contents in fresh samples are: young coconut water 0.102 g/100g; mature coconut water 0.028 g/100g; young coconut meat 1.529 g/100g; mature coconut meat 2.725 g/100g. NPN contents in dry samples are: young coconut water 0.18 g/100g; mature coconut water 0.13 g/100g; young coconut meat 0.28 g/100g; mature coconut meat 0.12 g/100g. NPN contents in fresh samples are: young coconut water 0.008 g/100g; mature coconut water 0.004 g/100g; young coconut meat 0.103 g/100g; mature coconut meat 0.117 g/100g. True protein contents in dry samples are: young coconut water 1.22 g/100g; mature coconut water 0.44 g/100g; young coconut meat 2.66 g/100g, mature coconut meat 2.22 g/100g. True protein contents in fresh samples are: young coconut water 0.056 g/100g; mature coconut water 0.011 g/100g; young coconut meat 0.981 g/100g; mature coconut meat 2.109 g/100g. The results show that total protein and NPN contents in young coconut water and meat are higher than in mature coconut water and meat.
Keywords: coconut water, coconut meat, protein, non protein nitrogen.

Introduction

In Indonesia, coconut palm is one of the biggest contributors for the economy of the people and nation. All parts of coconut fruit, from its outer shell to its meat, have some benefits. As food, coconut water and coconut meat contain high nutrients. The nutrients in coconut fruit have the same roles and functions as in the nutrients in other food substances. There are six nutrients that must be contained in foods, such as carbohydrates, proteins, fats, vitamins, mineral salts and water[1,2].

Coconut water is the liquid contained in coconut fruit. The main components are water, potassium, a small amount of carbohydrates, fats, proteins, and mineral salts. Nitrogen can be obtained from protein

contained in coconut water. Although it is low, protein in coconut water composed from 17 kinds of amino acids. Moreover, the protein content in coconut water is directly absorbable by the body. Coconut meat has some nutrients including proteins, fats, carbohydrates, calcium, phosphorous, iron, ascorbic acid, and water[3,4,5,6].

Proteins are one of a macronutrient group. Proteins have important role in the formation of biomolecules. However, if the organism is lack of energy, proteins can be used as energy source. The proteins are built up from simpler substances called amino acids, so free amino acids and other related simple nitrogenous compounds always occur in association with proteins[7,8].

The primary nutritional importance of protein is due to the fact that they are the major source of essential amino acids. The essential amino acids must be supplied in the diet either as free amino acids or as constituents of the dietary proteins. It is well known that proteins differ in their nutritive values due to the variability of amino acids composition, digestibility and availability of the digested proteins. To be absorbed, proteins must be broken down to individual amino acids or small peptides. The final results of the proteins digestion are mainly in the form of amino acids, and they are directly absorbed in 15 minutes after meals[9,10,11].

Changes in contents and chemical composition of coconut during maturation have been reported. The chemical composition changes in coconut water from six to nine months coconut fruits have been reported. The protein analysis results show that protein content decreases from 9.5 g/L to 6 g/L. The chemical composition changes in coconut meat have also been reported. Coconut meats were taken from four varieties of coconut fruit in maturation ranks as follows 5, 7, 9, 11, 13 and 14 months old. The analysis protein result shows that protein contents in four varieties of coconut meat increase at 7 months old but gradually decrease at 9 until 14 months old[12,13].

The aim of this study was to determine total protein and non protein nitrogen (NPN) changes in coconut water and meat from young and mature coconut fruit. The method used for analysis of total protein and NPN is Kjeldahl method.

Experimental

Apparatus

Apparatus used in this study were analytical balance, Kjeldahl flask, gas stove, water hose, liebig condenser, stative, clamps, Whatmann filter paper 42, hot plate, magnetic stirrer, aluminium cup, quartz sand, spatula, desiccator, oven, burette and laboratory glassware.

Materials

Coconut water and meat used in this study were derived from young and mature nuts of a coconut palm planted on a villager's garden located at Desa Saintis, Kecamatan Percut Sei Tuan, Kabupaten Deli Serdang, Medan. All chemical materials used in this study were 98% concentrated sulfuric acid, cupric sulfate, potassium sulfate, distilled water, 40% sodium hydroxide b/v, 0.02 N sulfuric acid, methyl red, methylene blue, phenolphthalein, 0.02 N sodium hydroxide and 10% trichloroacetic acid b/v.

Samples Preparation

Young coconut water: 20 g quartz sand and 20 g young coconut water were placed in a weighed aluminium cup, which then were dried in the oven at 70°C for 3 hr, cooled in the desiccator and weighed. The aluminium cup and sample were dried again in the oven for 30 min, cooled in desiccator and weighed. This treatment was repeated until constant weight (the difference of consecutive weighing is less than 0.2 mg) was achieved. 5 g of quartz sand and dry young coconut water mixture which was equal to 0.25 g dry young coconut water was then used for total protein and NPN determination in young coconut water[14].

Mature coconut water: 20 g quartz sand and 20 g mature coconut water were placed in a weighed aluminium cup, which then were dried in the oven at 70°C for 3 hr, cooled in the desiccator and weighed. The aluminium cup and sample were dried again in the oven for 30 min, cooled in desiccator and weighed. This treatment was repeated until constant weight (the difference of consecutive weighing is less than 0.2 mg) was

achieved. 10 g of quartz sand and dry mature coconut water mixture which was equal to 0.25 g dry mature coconut water was then used for total protein and NPN determination in mature coconut water[14].

Young coconut meat: 15 g young coconut meat were mashed and placed in a weighed aluminium cup, which then were dried in the oven at 70°C for the first 1 hr, then at 105°C for the next 2 hr, cooled in the desiccator and weighed. The aluminium cup and sample were dried again in the oven for 30 min, cooled in desiccator and weighed. This treatment was repeated until constant weight (consecutive weighing difference is less than 0.2 mg) was achieved. 0.25 g dry young coconut meat was then used for total protein and NPN determination in young coconut meat[14].

Mature coconut meat: 5 g mature coconut meat were mashed and placed in a weighed aluminium cup, which then were dried in the oven at 70°C for the first 1 hr, then at 105°C for the next 2 hr, cooled in the desiccator and weighed. The aluminium cup and sample were dried again in the oven for 30 min, cooled in desiccator and weighed. This treatment was repeated until constant weight (consecutive weighing difference is less than 0.2 mg) was achieved. 0.25 g dry mature coconut meat was then used for total protein and NPN determination in mature coconut meat[14].

Reagents Preparation

Reagents used in this study were 40% sodium hydroxide b/v, 0.02 N sulfuric acid, 0.02 N sodium hydroxide, 10% trichloroacetic acid (TCA) b/v, catalyst mixture of cupric sulfate and potassium sulfate, mixed indicator methyl red-methylene blue.

40% sodium hydroxide b/v was prepared by dissolving 40 g of sodium hydroxide pellets in 100 mL carbon dioxide-free distilled water. 0.02 N sulfuric acid was prepared by diluting 1.4 mL sulfuric acid 98% with distilled water in 1000 mL volumetric flask. 0.02 N sodium hydroxide was prepared by dissolving 0.8 g of sodium hydroxide with carbon dioxide-free distilled water in 1000 mL volumetric flask. 10% TCA b/v was prepared by dissolving 100 g of TCA in distilled water ad 1000 mL. Catalysts for digestion was prepared by mixing cupric sulfate and potassium sulfate (1:1). Mixed indicator methyl red-methylene blue was prepared by dissolving 100 mg of methyl red + 30 mg of methylene blue in 60 mL alcohol 95%, then diluted ad 100 mL boiled distilled water[14,15].

Standardization of Sodium Hydroxide Solution 0.02 N

0.1 g of oxalic acid ($C_2H_2O_4.2H_2O$) molar mass=126 was weighed and placed into 250 mL erlenmeyer flask, which then 25 mL distilled water was added. After it was dissolved, 2 drops of phenolphthalein indicator was added. Sodium hydroxide which would be standardized was then used for titration. The titration was done until the solution changed to pink[14].

$$\text{N NaOH} = \frac{\text{g oxalic acid x 2}}{\text{0,126 x mL NaOH}}$$

Determination of N-Total and Total Protein Content

Dry sample was weighed and placed into Kjeldahl flask, then 2 g of catalyst mixture and 3 mL of concentrated H_2SO_4 were added. Digestion was done for about 3 hr until the color of the digest is clear green. After it was cooled, 10 mL of distilled water was added and the solution was transferred into erlenmeyer flask. 15 mL of 40% NaOH were added to the solution until its color changed to black and then it was distilled. 25 mL of 0.02 N H_2SO_4 and 3 drops of mixed indicator were added into the receiving flask. The distillate were titrated with 0.02 N NaOH until the distillate changed from purple to green. Carry out a blank determination in the same way without the sample[14].

N-total content was calculate using the following expression:

$$\%\ \text{N (N-total)} = \frac{\text{mL NaOH (blank} - \text{sample)}}{\text{weight of sample (g) x 100}} \text{ x N NaOH x 14,008 x 100\%}$$

where, N NaOH = Normality of NaOH after standardization

Total protein content was calculated using the following expression:

$$\text{Total Protein (\%)} = \%\ \text{N-total x conversion factor}$$

where, conversion factor for coconut = 5.3.

N-total and total protein contents in fresh samples were calculated by converting N-total and total protein contents in dry samples to N-total and total protein contents in fresh samples.

Separation of Protein from Non Protein Nitrogen

Separation of protein from NPN was done by precipitating protein in the samples using 10% TCA. Dry sample was weighed and placed into 200 mL glass beaker. 50 mL of distilled water was added and allowed to stand for 30 min. 10 mL of 10% TCA was added, allowed to stand for 30 min, and then filtered. The precipitate which contained true protein was washed twice with TCA solution[17].

Determination of N-Protein and True Protein Content

True 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. 2 g of catalyst mixture and 3 mL of concentrated H_2SO_4 were added. The next procedure is as same as the procedure for total protein determination[17].

N-protein content was calculated by using the following expression:

$$\%\ \text{N (N-protein)} = \frac{\text{mL NaOH (blank} - \text{sample)}}{\text{weight of sample (g)} \times 1000} \times \text{N NaOH} \times 14{,}008 \times 100\%$$

where, N NaOH = Normality of NaOH after standardization
True protein content was calculated using the following expression:

$$\text{True Protein (\%)} = \%\ \text{N-protein} \times \text{conversion factor}$$

where, conversion factor for coconut = 5.3.

N-protein and true protein contents in fresh samples were calculated by converting N-protein and true protein contents in dry samples to N-protein and true protein contents in fresh samples.

Determination of Non Protein Nitrogen Content

NPN content was calculated by subtracting N-protein from N-total. NPN content in samples was calculated using the following expression:

$$\%\ \text{NPN} = \%\ \text{N-total} - \%\ \text{N-protein}$$

NPN 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 sample were analyzed using t test standard deviation method. Standard deviation was calculated using the following expression:

$$\text{SD} = \sqrt{\frac{\Sigma(X - \bar{X})^2}{n - 1}}$$

Data was rejected if $t_{value} \geq t_{table}$ at the confidence interval of 99% ($\alpha = 0{,}01$). t_{value} was calculated by using the following expression:

$$t_{value} = \left|\frac{X - \bar{X}}{SD\sqrt{n}}\right|$$

where, SD = Standard deviation
X = Protein content

$\bar{X}$ = Mean of the protein content
n = number of determinations
The actual protein content was calculated using the following expression:

$$\mu = \bar{X} \pm t_{table} \times \frac{SD}{\sqrt{n}}$$

where, μ = Actual protein content
$\bar{X}$ = Mean of the protein content
SD = Standard deviation
n = number of determinations[18,19].

Results and Discussion

Sample Identification Result

The sample identification result shows that samples used for analysis are coconut fruit (*Cocos nucifera* L.) from familia Arecaceae.

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

The results of N-total, N-protein, total protein, true protein and NPN determination in young coconut water, mature coconut water, young coconut meat and mature coconut meat are shown in Table 1.

Table 1. N-Total, N-Protein, Total Protein, True Protein and Non Protein Nitrogen Contents in Samples

Sample	Kadar (g/100g)				
	N-Total	N-Protein	Total Protein	True Protein	NPN
Young Coconut Water	0,41±0,0666[a] 0,019±0,0034[b]	0,23±0,0285[a] 0,011±0,0018[b]	2,19±0,3439[a] 0,102±0,0156[b]	1,22±0,1563[a] 0,056±0,0076[b]	0,18[a] 0,008[b] 43,90[*]
Mature Coconut Water	0,21±0,0285[a] 0,006±0,0013[b]	0,08±0,0232[a] 0,002±0,0023[b]	1,13±0,1362[a] 0,028±0,0033[b]	0,44±0,1130[a] 0,011±0,0018[b]	0,13[a] 0,004[b] 61,90[*]
Young Coconut Meat	0,78±0,0360[a] 0,288±0,0127[b]	0,50±0,0750[a] 0,185±0,0276[b]	4,14±0,1974[a] 1,529±0,0732[b]	2,66±0,3986[a] 0,981±0,3286[b]	0,28[a] 0,103[b] 35,90[*]
Mature Coconut Meat	0,54±0,0390[a] 0,514±0,0370[b]	0,42±0,0441[a] 0,397±0,0418[b]	2,87±0,2076[a] 2,725±0,1912[b]	2,22±0,2183[a] 2,109±0,4636[b]	0,12[a] 0,117[b] 22,22[*]

Each value represents an average of six determinations (n=6)
[a]: content in dry sample
[b]: content in fresh sample
[*]: content expressed as percent of total nitrogen in sample

Total protein and true protein content in young and mature coconut water are significantly different, as well as in young and mature coconut meat. Total protein content measured on dry weight in young coconut water (2.19 g/100g) is higher than in mature coconut water (1.13 g/100g), likewise, total protein content in young coconut meat (4.14 g/100g) is higher than in mature coconut meat (2.87 g/100g). True protein obtained after separation from NPN decreases. True protein in young coconut water (1.22 g/100g) is higher than in mature coconut water (0.44 g/100g), as well as true protein in young coconut meat (2.66 g/100g) is higher than in mature coconut meat (2.22 g/100g).

Total protein, true protein, and NPN content in young coconut meat is lower than in mature coconut meat if measured on fresh samples, this is due to the higher water content in young coconut meat than in mature

coconut meat. From statistical test results, total protein and true protein contents in fresh young and mature coconut water are significantly different, as well as in young and mature coconut meat.

The higher total protein content in young coconut water and meat than in mature coconut water and meat is in the agreement with the study conducted by Vigliar et al. (2006) that protein content in coconut water decreases from 9.2 g/L to 6 g/L as the coconut fruit matures, and also in agreement with Kurian and Peter (2009) that protein content in coconut meat increases at 7 months old and then decreases gradually until 12 months old. Futhermore, the study conducted by Assa et al. (2010) also shows that total protein content in coconut meat from 4 varieties of coconut palm decrease during nuts maturation. The decreasing of protein content in coconut water and meat is probably caused by the increasing of other nutrients like fats and carbohydrates which increase more rapidly than the proteins, thus protein content in mature coconut water and meat is lower[12,13,20].

In this study, NPN content obtained in young coconut water (0.18 g/100g) is higher than in mature coconut water (0.13 g/100g), and in young coconut meat (0.28 g/100g) is higher than in mature coconut meat (0.12 g/100g). The decreasing of NPN content is probably caused by the changes of free amino acids to form proteins during nut maturation.

In most foods, 95% of total nitrogen exists as protein and free amino acids, this can be considered as a way to measure amino acids content. This shows that free amino acids content in young coconut water and meat is higher than in mature coconut water and meat, thus young coconut water and meat is better to be consumed as amino acids source because free amino acids is easier to be absorbed by the gastrointestinal tract[21].

Most proteins are digested into amino acids, the rest are digested into tripeptide and dipeptide. Protein digestion or hydrolisis begins in the stomach by chloride acid and pepsin. Because food stay in stomach for a short time, proteins are only digested to polypeptide, proteose, and pepton mixture. The digestion continues in small intestines by mixture of protease enzyms like trypsine, chymotrypsine, carboxypeptidase, and elastase. These enzyms break the protein from polypeptide to small peptides like tripeptide, dipeptide and amino acids. Proteolytic enzyms in stomach and small intestines finally digest most proteins in foods into free amino acids. The end results of the protein digestion which mainly in form of amino acids are then absorbed directly in 15 minutes after meals[11].

If expressed as percent of total nitrogen, it turns out that NPN content is considerable. The NPN content expressed as percent of total nitrogen from the highes to the lowest are mature coconut water (61.90 g/100g), young coconut water (43.90 g/100g), young coconut meat (35.90 g/100g) and mature coconut meat (22.22 g/100g). 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. They provide a source of nutrients and growth factors that are important in malting, brewing and panary fermentation[22].

Conclusion

Total protein contents in dry samples are: young coconut water 2.19 g/100g; mature coconut water 1.13 g/100g; young coconut meat 4.14 g/100g; mature coconut meat 2.87 g/100g. Total protein contents in fresh samples are: young coconut water 0.102 g/100g; mature coconut water 0.028 g/100g; young coconut meat 1.529 g/100g; mature coconut meat 2.725 g/100g.

NPN contents in dry samples are: young coconut water 0.18 g/100g; mature coconut water 0.13 g/100g; young coconut water 0.28 g/100g; mature coconut meat 0.12 g/100g. NPN contents in fresh samples are: young coconut water 0.008 g/100g; mature coconut water 0.004 g/100g; young coconut meat 0.103 g/100g; mature coconut meat 0.117 g/100g.

True protein contents in dry samples are: young coconut water 1.22 g/100g; mature coconut water 0.44 g/100g, young coconut meat 2.66 g/100g, mature coconut meat 2.22 g/100g. True protein contents in fresh samples are: young coconut water 0.056 g/100g; mature coconut water 0.011 g/100g; young coconut meat 0.981 g/100g; mature coconut meat 2.109 g/100g.

The results obtained in this study show that the total protein and true protein contents in young and mature coconut water is significantly different, as well as in young and mature coconut meat. Total protein, NPN and true protein contents in young coconut water are higher than in mature coconut water determined on either fresh or dry samples. Total protein, NPN and true protein contents in young coconut meat are higher than in mature coconut meat determined on dry samples, while on fresh samples, the contents in young coconut meat are lower than in mature coconut meat.

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