International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.12, pp 543-549, 2016

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

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Abstract : Pakkatis atraditional foodinMandailingNatalis taken fromthe inside of theyoungrattan and the most commonly consumed by people is grilled pakkat.Pakkatcan beused as vegetablesandbelieved to curemany diseasesthatneed to be researchedcontent ofnutrientsin it and is expected totake itbecomeone of thefunctional 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.Thesamples used in this study is young rattan from the forest of LumbanPasir 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 pakkat are 0.600 g/100g,0.488 g/100g and 0.315 g/100grespectively. 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 veryinterested because it is believed to cure various diseases such as diabetes and malaria, also have efficacy as a whet your appetite when fasting, so thatpakkatis expected tobe usedas afunctional food. Functional foods as any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains[1,2].

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[3,4].

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 essensial source of amino acid in the dict which are necessary for normal growth and maintenance of the body[5]. Food proteins have two mayor 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 ordefense mechanism of the body[5,6].The Kjeldahl and visble spectrofotometry method commonly used for analysis total protein and non protein nitrogen[7,8].

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 inthis 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 identificationof plantis donein theHerbariumMedanense, HerbariumLaboratoryFaculty of Mathematicsand Natural Sciences, University ofNorth Sumatera.

Chemical materials

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

Samples Preparation

Freshpakkat: youngrattanhas been cleared taken part inwhiteand then blended, and weighed1g fresh pakkat to be used for total protein and NPN determination.

Grilled pakkat: young rattan hasgreenouter skinis grilledusingfirewoodfor ±15minutesuntilthe outer skincoloured black, cooling, take part in white young rattanandblended, weighed1g grilled pakkattobe usedfor totalprotein and NPN determination.

Boiled pakkat: young rattan has been cleared taken part inwhite, boiling with boiled waterat 100°Cwith a ratio boiled pakkatas much as100 gandthe water used 500ml for ±15minutes until pure white with a boiling. Cooling and blended and then weighed1g boiled pakkat to be usedfor totalprotein and NPN determination.

Standardization of0.01NHydrochloricAcidSolution

0,01gof sodium tetraborate ($Na_2B_4O_7.H_2O$) was weighed then placed into100 ml erlenmeyer and10ml distilled water. After soluble, added 2drops ofindicator 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[9].

$$N\ HCl = \frac{Na2B4O7\ (g)}{Equivalen\ weight\ (Na2B4O7\ )\ x\ V\ HCl\ (ml)}$$

Determination ofWater Content

Determination of water contentwas done by Gravimetry method. Fresh pakkat has been blended, then weighed quickly as much as2g into a porcelain crucible of known weight and was dried for30 minutes at105ºC. Leveled by shaking slowly, inserted into the oven at105ºCfor 3hours and cooling in desiccator and weighed. Repeat the heating, cooling and weighing until a constant weight[10]. Weighing the samples in water content can be expressed by wet basis or dry basis.

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

Where : a). weight ofsamplesbeforebe dried, b). loss ofweightafterbe 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 a100 ml of flask, matched up tothe mark with distilled water. Pipette25ml of solution and add 50ml of 40% sodium hydroxide was put into the distillation apparatus.25ml of4% boric acid and 3 drops of indicator mengsel were added into the receiving flask. The destillate were titrated with0.01 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[10].

Determination of total protein content was conducted ona 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:

$$\%\ N\text{-total} = \frac{Volume\ (ml)\ HCl\ (sample\text{-}blank)}{weight\ of\ sample\ (g)\ x1000} x\ N\ HCl\ x\ 14.007\ x\ Dilution\ Factor\ x100\%$$

where, N HCl = 0.0117 N

Total protein content was calculated using the following expression:

$$Total\ Protein\ (\%) = \%\ N\text{-total}\ x\ Conversion\ Factor$$

where, conversion factor for pakkat = 6.25[9].

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[11].

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 concentratedsulfuric acid were added. The next procedure is as same as the procedure for total protein determination[10].

N-protein content was calculated using the following expression:

$$\% \text{ N-protein} = \frac{\text{Volume (ml) HCl (sample - blank)}}{\text{weight of sample (g) x1000}} \text{x N HCl x14.007 x Dilution Factor x100\%}$$

where, N HCl = 0.0117 N

Pure protein content was calculated using the following expression:

$$\text{Pure Protein (\%)} = \% \text{ N-total x Conversion Factor}$$

where, conversion factor for pakkat = 6.25[9].

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:

$$\text{NPN content as percent of N-Total (\%)} = \frac{\% \text{ NPN}}{\% \text{ N-total}} \text{ x 100\%}$$

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:

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

Data was rejected if t value ≥ 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:

$$\mu = \bar{X} \pm t_{tabel} x \frac{SD}{\sqrt{n}}$$

where,μ = Actual protein content

$\bar{X}$ = Mean of the protein content

SD = Standard deviation

n = 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.

OrganolepticSamples

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

Table 1.Organolepticobservations in pakkat

Description	Fresh Pakkat	Grilled Pakkat	Boiled Pakkat
Diameter	1.2 cm	1.2 cm	1.2 cm
Outer skin	Green	Black	Green
Pakkat colour	White	White	Pure white color
Taste	Extremely bitter (+++++)	Bitter (+++)	Bitter (+++)
Texture	Hard	Soft	Soft

Watercontent in thesamples

Determination ofwater contentin thesamples was done byGravimetrymethod. The water contentinfresh, grilledandboiledpakkatobtained can beseen in Table2 below:

Table2.Results ofWater ContentinFreshPakkat, Grilled Pakkatand Boiled Pakkat

Samples	Water Content (%)
Fresh Pakkat	88.68
Grilled Pakkat	88.25
Boiled Pakkat	90.66

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 becausethe 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[14].

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/100 g and 1.79 g/100g, and the boiled pakkat 3.07 g/100 g 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 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

Samples	Content (g/100g)				
	N-Total	N-Protein	Total Protein	Pure Protein	NPN
Fresh Pakkat	0.961±0.0025	0.361±0.0016	6.00±0.0165[a] 53.03±1.1156[b]	2.25±0,0104[a] 19.91±0,4114[b]	0.600 62.43[*]
Grilled Pakkat	0.775±0.0031	0.287±0.0013	4.82±0.0232[a] 41.25±0.247[b]	1.79±0.0104[a] 15.26±0.2471[b]	0.488 62.97[*]
Boiled Pakkat	0.491±0.0025	0.176±0,0043	3.06±0.0194[a] 32.9±0.5095[b]	1.10±0.0265[a] 11.76±0.2706[b]	0.315 64.15[*]

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 boiledprocess[5].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[15].

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 inrecent 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 foodattributes[16].

Conclusion

Total protein content were obtained on a samplesof fresh, grilled and boiled pakkat are 6.00g/100g, 4.84g/100g and3.07g/100 g recpectively. NPN levels obtained in samples of fresh, grilled and boiled are 0.600g/100g, 0.488g/100g and 0.315g/100g recpectively. 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.

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