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Protein quality of foods made incorporating *Cycas circinalis* seed flour

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Abstract

Among the cycad species *Cycas circinalis* and *Cycas zeylanica* are native to Sri Lanka. Foods such as *pittu* and *roti* made by incorporating *Cycas circinalis* seed flour are consumed by Sri Lankans living in areas where the plant grows. Although previously reported data indicate that the content of protein in *C. circinalis* seeds is comparable to that of wheat flour, no data is available on the quality of cycad seed proteins. The present study was aimed at evaluating the quality of proteins in *C. circinalis* seed flour, *pittu* and *roti* made by incorporating cycas seed flour.

The moisture, insoluble (IDF) and soluble dietary fibres (SDF), crude protein and digestible carbohydrate contents were measured by AOAC official methods (1984), method of Asp *et al*, Lowry's method/ Folin Ceocalteau method and Holm's method respectively. Quality of proteins was estimated by AOAC Official method 960.48, using an animal bioassay.

Digestible carbohydrate content was highest in soaked seed flour (50.0±2.03). Among the cycad foods, highest protein content (10.3±0.01) and total dietary fibre content (7.7) were in *roti* and this could be due to the addition of wheat flour to *roti* instead of rice flour in *pittu*. Highest weight gain was observed in reference group fed with casein containing diet. The significantly high weight gain in the seed flour fed group compared to *roti* and *pittu* fed groups may be due to the high feed intake. No significant weight gain difference was observed between *roti* and *pittu* fed groups. Protein quality of *C. circinalis* seeds was comparable with that of common maize but all protein quality parameters [Protein Efficiency Ratio (PER), Food Efficiency Ratio (FER), Net Protein Retention (NPR) and Protein Retention Efficiency (PRE)] were significantly low compared to the reference protein diet. Although not significant, NPR and PRE values in *roti* (1.4±0.6 and 8.5±3.7) were higher than test food made with *pittu* or raw seed flour.

Key words: protein quality, Protein Efficiency Ratio (PER), Food Efficiency Ratio (FER), Net Protein Retention (NPR) and Protein Retention Efficiency (PRE)

Introduction

Cycas seeds are a popular food among the people in some parts of the world and the common names such as wild pineapple (*Macrozomia*), wild date (*Encephalartos*), wild sago (*Zamia floridana*), false sago palm (*C.circinalis*), bread palm (*C. rumphii*) and sago palm (*C. revoluta*) etc. applied to the varieties of this plant reveals the profuse usage of these seeds as a food. Even at present, cycad seeds are the staple food of the people in Guam, Irian Jaya and Kii peninsula and the seeds are also used for medicinal and recreational purposes (Spencer, 1990).

Among cycad species, *Cycas circinalis* and *Cycas zeylanica* are native to Sri Lanka with growth restricted to certain parts of the island (Hill, 2004) and the seeds are consumed as a food by the residents of these areas. Since the distant past, *C. circinalis* seed flour has been used by the Sri Lankans in the preparation of two starchy staples, "*pittu*" and "*roti*".

A preliminary study has revealed that crude protein content in *Cycas circinalis* seeds is comparable to that of wheat (Senevirathne, 2009). Further, according to folklore, consumption of foods made from *Cycas circinalis* is considered as a remedy for Protein Energy Malnutrition (PEM) in Sri Lanka. However, the protein nutritional quality of the seeds had not been scientifically investigated.Therefore the objective of the present study was to estimate the proximate composition and to evaluate the protein quality of *Cycas circinalis* seed flour and common foods made incorporating seed flour.

Materials and Methods

Materials

Cycas circinalis seeds (10 kg) were purchased from a whole sale dealer in Colombo, Sri Lanka. Wheat flour and rice flour used for food preparation were purchased from a retail outlet in Colombo, Sri Lanka. The mature scraped coconuts (8-10 months maturity) were used in the preparation of *pittu* and *rotti*. Vitamin and mineral mix for the animal bioassay was purchased from Hexagon Nutrition (Pvt) Ltd., Nasik, India.

Preparation of the samples and food

Preparation of unprocessed seed flour:

Dried *C. circinalis* seeds were soaked (0.2 Kg /L water for 12 hours) and excess water drained. The seeds were sun dried until hard in consistency and made in to flour (particle size 0.3 – 0.5 mm).

a) *Pittu* preparation: Above seed flour, rice flour and scraped coconut were mixed in 1:1:0.7 ratio with salt to taste. The mixture was then loosely packed in to a columnar vessel (*pittu bamboo*) and steamed cooked for 10 min (wet heat).

b) *Roti* preparation: Above seed flour, wheat flour and scraped coconut (1:1:0.7) were mixed and made into a dough with adequate water and salt. The dough (50g) was then flattened (diameter 15 cm and the thickness 0.5 cm) and cooked on a heated flat pan by turning sides every 2 min for 10 min (dry heat).

The oven dried foods from a) and b) were made into flour by milling and sieving through a 0.3-0.5 mm mesh, and with seed flour from above were used to determine the macronutrients and dietary fibre. Seed flour (raw), milled *pittu* and *roti* were incorporated in to the animal diets.

Estimation of proximate compositions

The moisture content was measured by AOAC official method (1984) 14.004. Insoluble (IDF) and soluble dietary fibres (SDF) were determined by the method of Asp (Asp, 1983). The crude protein was by Lowry's method/ Folin Ceocalteau method (Lowry *et al*, 1951). The digestible carbohydrate content was measured by Holm's method (Holm *et al*, 1986).

Animal model

Twenty five ICR laboratory male mice, aged 28 days from the same colony, were obtained from Medical Research Institute, Colombo, Sri Lanka. Animals were kept under standard conditions that would not interfere with normal growth. On arrival mice were placed in separate cages and the standard mice diet (Subourdy, 1988) and water *ad libitum* provided for 3 days for acclimatization. On the fourth day the mice were divided in to 5 groups (5 mice/ group) so that the weights of mice in each group was not significantly different ($P<0.05$) and the difference between the mean weights of the groups did not exceed 5g. The five groups were basal, reference and three test diet groups.

Preparation of diets

The three test diets and reference diet were prepared in order to provide 10% protein from each diet. Soaked unprocessed seed flour diet, *pittu* diet and *roti* diet were prepared with soaked unprocessed seed flour 850 g/ 1 kg food, *pittu* flour 910 g/ 1 kg food and *roti* flour 816 g/ 1 kg food respectively. Glucose, corn starch, cellulose, corn oil, vitamin and mineral mix were the other ingredients incorporated in to the diets. The ingredients were adjusted so that digestible carbohydrate, total fibre, fat and protein contents in all diets were similar and diets were iso caloric. Diet for basal group was prepared with all the ingredients as in other diets except protein.

Evaluation of protein quality

The three test groups were provided with the appropriate test diets according to AOAC Official method 960.48 (10g/ mouse/ day). Reference group was provided with a standard diet with casein supplemented with L-methionine (10g/day/mouse). The basal group was provided a diet without protein. Vitamin mix contained vitamin A, D, E, menadione, choline, p-aminobenzoic acid, inositol, niacin, Ca, pantothenate, riboflavin, thiamin HCl, pyridoxine HCl, folic acid, biotin and vitamin B_{12}. Mineral mix was composed of NaCl, KI, KH_2O_4, $MgSO_4$, $CaCO_3$, $FeSO_4.7H_2O$, $MgSO_4.H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$ and $CoCl_2.6H_2O$. The reference, basal and the test diets (10g/day/mouse) were fed to mice for 28 days.

Throughout the study, the weights of the mice were measured once in three days and feed intake recorded daily. The urine and feces output was observed daily.

Protein Efficiency Ratio (PER), Food Efficiency Ratio (FER), Net Protein Retention (NPR) and Protein Retention Efficiency (PRE) were calculated using the following equations to ascertain the protein quality.

PER = Weight gain/ Protein intake
FER = Weight gain of test animal (g)/ weight of the food consumed (g)
NPR = Weight gain of test animal (g) – weight loss of protein free basal group/ weight of test protein consumed (g)
PRE = NPR x 6

Statistical analysis

Proximate data were analyzed using Microsoft Excel 2000. The differences between the means of the groups were analyzed by ANOVA using SPSS version 13.

Ethical clearance

Ethical approval for the study was obtained from the Ethics Rewiev Committee of the Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka (Approval number 431/09).

Results

The moisture, digestible carbohydrate, protein, SDF and IDF contents are stated in Table 1. The moisture content of freshly cooked *pittu* was higher than that of *roti*. Digestible carbohydrate contents were significantly different among *pittu, roti* ($p \leq 0.05$) and seed flour with seed flour having the highest digestible carbohydrate (50.0±2.03). The protein contents in soaked seed flour, *pittu* and *roti* flour ranged from 9.1% - 11.9% ($p \geq 0.05$).

Table I: Proximate compositions of *Cycas* seed flour and pittu and roti made of *Cycas* seed flour

% of nutrient	*Seed flour*	*Pittu flour*	*Roti flour*
Moisture	7.4±0.51^{a}	43.7±0.01^{a}	29.0±0.03^{b}
*Carbohydrate**	50.0±2.03^{a}	31.6 ±1.11^{b}	36.3±2.15^{c}
*Protein**	10.0±0.01^{a}	9.3±0.03^{a}	10.3±0.01^{a}
*IDF**	10.7±1.73^{a}	4.1±0.19^{b}	5.1±0.17^{c}
*SDF**	4.6±0.40^{a}	1.8±0.23^{b}	2.6±0.22^{b}

N=6; *Dry weight basis; Same superscript along a row indicates no significant difference ($p=0.05$)

IDF and SDF amounts were significantly high ($p \leq 0.05$) in seed flour indicating the presence of higher amount of fibre in *Cycas* seeds compared to the added coconut-wheat flour to *roti* and coconut-rice flour to *pittu*. However, compared to *roti*, total fibre content in *pittu* was significantly lower ($p \leq 0.05$).

During the assay period, an obvious weight loss was perceived in the basal group whereas a significant weight gain was observed in the reference group (Table II and figure 1). Mice fed diets formulated with *roti* and *pittu* had similar weight gain ($p \geq 0.05$). However, the group fed soaked seed flour diet demonstrated a significant weight gain ($p \leq 0.05$) compared to the other two test groups with a higher feed intake than other groups. Although the weight gains between *pittu* and *roti* fed groups were not significantly different ($p \geq 0.05$), *roti* fed group achieved the same weight by consuming a lesser amount of food than the *pittu* fed group.

Table II: Feed intake and mean weight changes in basal, reference, flour, pittu and roti fed mice groups

Group	*Total feed intake*	*Mean weight gain*
Basal	122	-6.4
Reference	463	15
Flour	827	9.6
Pittu	520	4.8
Roti	353	5

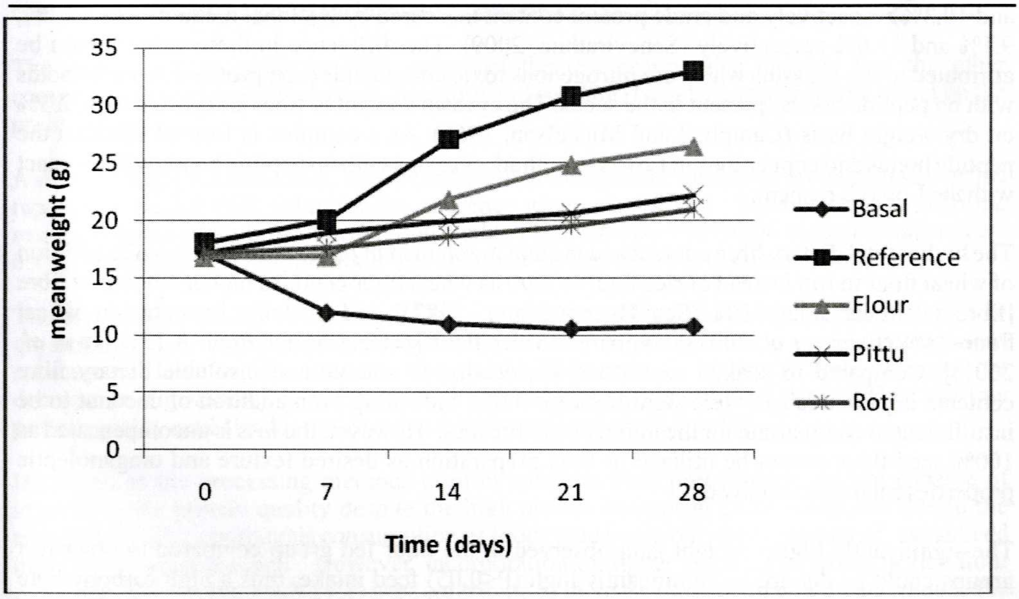

Figure 1: Mean weight gain/ loss of the mice in different groups

Table III indicates the protein quality parameters of the reference and test diets. All protein quality parameters were significantly lower ($p \leq 0.05$) in all test groups than reference group, however there was no significant difference in these parameters among the test groups.

Table III: Protein quality of reference, flour, pittu and roti diets

Group	*PER*	*FER*	*NPR*	*PRE*
Reference	1.6±0.2	0.16±0.02	2.3±0.3	14.0±1.6
Flour	0.6±0.1 ^{a}	0.06±0.01 ^{a}	1.0±0.1 ^{a}	5.8±0.7 ^{a}
Pittu	0.4±0.2 ^{a}	0.04±0.03 ^{a}	1.1±0.2 ^{a}	6.6±1.2 ^{a}
Roti	0.5±0.7 ^{a}	0.05±0.07 ^{a}	1.4±0.6 ^{a}	8.5±3.7 ^{a}

Same superscript along a row indicates no significant difference ($p \leq 0.05$)

Discussion

Higher moisture content was obtained for wet heat processed freshly cooked *pittu* whereas *roti* prepared by dry heat had lower moisture content. The significantly low ($p \leq 0.05$) digestible carbohydrate in *pittu* and *roti* than in seed flour is due to the addition of other ingredients to these foods (i.e. scraped coconut). According to literature, the digestible carbohydrate contents in wheat (83.4%) and rice flour (85.3%) (Thadhani *et al*, 2000) is comparable. However, in the present study, significantly higher digestible carbohydrate content was obtained for *roti* (wheat) than *pittu* (rice) which could be due to underestimation of carbohydrate in *pittu*.

According to Lowry's method, the protein content in seed flour, *pittu* and *roti* were 10.0%, 9.3% and 10.3% respectively and crude protein content measured by Kjeldhal method were 12.1%, 9.1% and 11.6% respectively (Senevirathne, 2009). The difference in these values could be attributed to the cycasins which are nitrogenous toxic compounds (non proteinous amino acids with no peptide bonds) present in the seeds. The cycasin content in flour is reported to be 2.3% on dry weight basis (Campbell and Mickelson, 1966). As a complex is formed between the peptide bonds and copper ions in Lowry's method, cycasins with no peptide bonds will not react with the Lowry's reagent.

The higher total dietary fibre content and protein in *roti* than in *pittu* could be due to the addition of wheat flour to *roti* instead of rice flour to *pittu* as wheat flour contains higher amount of fibre [fibre in wheat flour~10% (San Buenaventura, 1987)] and proteins [proteins in wheat flour~14% (Reese *et al*, 2007)] compared to rice flour [protein in rice flour~8.75% (Ju *et al*, 2001)]. Compared to soaked seed flour, the decline in soluble and insoluble dietary fibre contents in *pittu* and *roti* were significant ($p<0.05$), indicating even addition of coconut to be insufficient to compensate for the indigenous fibre loss. However, the loss is uncompensated as 100% seed flour cannot be utilized in food preparation as desired texture and oraganoleptic properties cannot be achieved.

The significantly higher weight gain observed in the flour fed group compared to other test groups could be due to the significantly high ($P<0.05$) feed intake, thus a high carbohydrate content (827g) (Table 2). As weight gain of unprocessed flour fed group was not significantly proportionate to the feed intake when compared to the *pittu* and *roti* fed groups, the weight gain of unprocessed seed flour group could be mainly due to the to be completed The lowest feed intake (353 g) was observed in the group fed the test diet made incorporating *roti* flour and a decrease in feed intake with time was observed.

The low feed intake in animals in *roti* and *pittu* diets fed groups may have been due to undesirable organoleptic properties as it is known the feed intake decrease in such instances (Sulabo *et al*, 2008). The significant weight loss in basal group was due to low feed intake and lack of protein in their diet.

PER and FER are indicators of weight gain of the animals according to the amount of protein and food consumed respectively. NPR is the actual protein retention of the test animals considering the contribution of the protein in food for both the weight gain and normal body functions [NPR = (weight gain of test – weight loss of basal)/ weight of protein consumed]. The amount of nitrogen retained in the body after consumption of the food is expressed by PRE.

FER, PRE, NPR and PER (Table 3) of all test groups were significantly low when compared with the reference group ($P<0.05$) indicating that the quality of protein in all test groups are low. When considering the FER the high intake of soaked flour and *pittu* flour incorporated diets had not contributed to an increase in the weight gain compared to the reference. Similarly the low PER of all test diets indicate that the protein has not contributed to a significant weight gain due to consumption of test diets. The low NPR and PRE indicate low nitrogen retention in the body due to the imbalance of dispensable and indispensable amino acid thus low quality proteins in the test diets.

However, when comparing the feed intakes, protein quality and weight gain, the *roti* diet fed mice with the lowest feed intake among test groups had a similar protein quality to unprocessed and *pittu* flour diets. This indicates that dry processing improves protein quality than wet processing (*pittu*). The incorporation of wheat flour with a higher protein could have also contributed to the increase in protein quality in *roti*. Another reason could be the consumption of less amount of cycas seed toxins by *roti* fed group due to low feed intake [unprocessed flour fed group, *pittu* fed group and *roti* fed group had consumed 1.3g of cycasin/ mouse, 0.9g of cycasin/ mouse and 0.5g of cycasin/ mouse respectively].

The statistically insignificant FER values of the test groups also indicate that the other components in *roti* and *pittu* (wheat, rice flour and coconut) have had no significant effect on the protein quality.

A similar study (Williams *et al*, 2008) conducted to estimate the quality of proteins in maize had reported a similar PER value for the reference group (1.400.13). When compared with the results of the same study (first generation quality protein maize = 0.970.06 and common maize = 0.480.28), PER of *C. circinalis* flour was comparable with the PER of common maize.

The low protein quality in cycas seed flour could be attributed to many factors such as low digestibility, low indispensable amino acids and the presence of non protein nitrogenous substances. However, the absence of trypsin inhibitors in *C. circinalis* flour indicates the decrease in digestibility is not due to enzyme inhibition (unpublished data). Thus the low protein quality could be due mainly to an imbalanced amino acid profile.

However, as the processing methods used in common food preparations are not capable of improving the protein quality despite the high protein content in cycas seeds and due to the reported toxicity, continuous consumption of foods made with only/high amount of cycas seed flour is not recommended. However, incorporation of higher amounts of wheat or rice flour other than used in this study (1:1) may contribute to increase the protein quality and reduce the toxicity in diets.

Conclusions

The quality of proteins in cycas seed flour is comparable to that of common maize. The processing methods used in the current study or the ingredient ratios were unable to contribute to significantly increase the protein quality compared to raw seed flour. However, dry heat treatment improved the protein quality than wet heat treatment. When considering the nutritional aspects, addition of other ingredients to cycas seed flour in the ratios in the present study was only marginally beneficial.

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References

AOAC (1984) Methods of Association of Analytical Chemists, 14.004, Virginia, DC USA.

AOAC (2000) Methods of Association of Analytical Chemists, 960.48, Virginia, DC USA.

Asp, N.G., Hallmerh, C.G., Siljestrom, M. (1983) Rapid enzymatic assay for insoluble and soluble dietary fibre. *Journal of Agriculture Food Chemistry,* 31, 476-482.

Campbell, M.E., Mickelson, O. (1966) Effects of Strain, Age and Diet on the Response of Rats to the Ingestion of *Cycas circinalis. Journal of Nutrition,* 99, 115-117.

Hill, K. (2004) Introduction to Cycads (Published by Royal Botanical Gardens, Sydney)[Internet] Sydney: Available at: http://plantnet.rbgsyd.nsw.gov.au/PlantNet/cycad/index.html [Accessed on 20th November 2008]

Holm, J., Bjorck, D.M., Drews, A., Asp, N.G.(1986) A rapid method for analysis of starch. *Starch/Starke*, 38, 224-226.

Ju, Z.Y., Hettiarachchy, N.S., Rath, N. (2001) Extraction, Denaturation and Hydrophobic Properties of Rice Flour Proteins. *Journal of Food Chemistry and Toxicology*, 66(2), 229-232.

Lowry, O.H., Rosebrough, N.J. *et al.*(1951) Lowrys method - The original Method. *Journal of Biological Chemistry*, 193, 256.

Reese, C. L., Clay, D. E., Beck, D., Englund, R. (2007) Is protein enough for assessing wheat flour quality?, Western Nutrient Management Conference, 7, 85-90.

San Buenaventura, M. L., Dong, F. M., Rasco, B. A. (1987) The Total Dietary fibre content of Wheat, Corn, Barley, Sorghum and Distillers' Dried Grains with Solubles. *Journal of Cereal Chemistry*, 64(2), 135-136.

Senevirathne, I. (2009) Nutritional values and chemical composition of some traditional foods in Sri Lanka., Theses

Spencer, P.S. (1990) Are Neurotoxins Driving Us Crazy? Planetary Observations on the causes of Neurodegenerative Diseases of old age, Behavioral Measures of Neurotoxicity, Report of a Symposium, Washington, D.C., 11-36

Subourdy, M.A. (1988) Breeding and care laboratory animals. WHO/ Lab/88.1.1: 45.

Sulabo, R. C., Tokach, M. D., DeRouchey, J. M., Risley, C. D., Nelssen, J. L., Dritz, S. S., Goodband, R. D. (2008) Influence of organoleptic properties of the feed and nursery diet complexity on preweaning and nursery performance, Swine Day 2008, 32-41.

Thadhani, W. M., Jansz, E. R., Peiris, H. (2000) The Digestible Carbohydrate and Dietary Fibre content of common Sri Lankan Plant Food stuff. *Journal of National Science Foundation of Sri Lanka*, 28(4), 253-264.

Williams, I.O., Agiang, M.A. *et al.* (2008) Nutritional Quality of 1st Generation quality protein Maize die and its Effect on some Biological indices of Albino Wistar rats. *American Journal of food technology*, 1557-4571.

Wilson, K., Walker, J. (2000) "Practical Biochemistry: Principles and techniques", Cambridge University Press.