Nigerian Journal of Biochemistry and Molecular Biology
Volume 16, 2001. Number 1 (Printed in Nigeria)

0189-4757/96$3.00+0.00

NJBMB 2047

Studies on Some Antinutritive Factors and *In-Vitro* Protein Digestibility of *Thaumatococcus danielli* (Benth) Waste

B. O. Elemo*, G. N. Elemo, O. O. Agboola and A. B. Oyedun
Department of Biochemistry, Lagos State University, P. M. B. 1087, Apapa, Lagos, Nigeria.

ABSTRACT

The waste generated from the extraction process of thaumatin, an intensely sweet protein from *Thaumatococcus danielli* is about 99.9% (w/w) of the whole fruit. Nutritional assessment of this waste was carried out to identify its possible use as animal feedstuff.

Levels of phytic acid, polyphenols, tannins and saponins in the pericarp and seed of *T. danielli* were determined. Sequential extraction, solubility profile and in vitro digestibility of *T. danielli* proteins were also carried out.

T. danielli pericarp contained 4.10mg/g tannins, 22.67 mg/g phytic acid, 224.17mgIg polyphenols and O.6mg/g saponins. The seed contained 7.5mg/g tannins, 25.67mg/g phytic acid, 61.67mg/g polyphenols and O.74mg/g saponins.

Preliminary solubility profile of *T. danielli* proteins showed dilute alkali to be one of the best solvents for protein extraction of both pulp and seed (O.5M NaOH and 1.0M NaOH respectively). From the sequential protein extraction, glutelins were highest in both seed and pulp *(45%* and 40% respectively), while albumins were the least with 8% and 1% respectively of the extractable protein. Protein digestibility was low in both pericarp and seed (44.6% and 38.9% respectively).

INTRODUCTION

Inadequacy of food supply in developing countries has been linked among other things to the large amount of post harvest food loss during storage, handling and processing (NAS, 1978). Much of the agricultural waste generated during processing could have been salvaged if information on their uses were readily available.

Meeting the protein needs of majority of the population can be achieved directly by supplying them with protein rich foods or indirectly, by the provision of cheap and readily available nutrient rich feeds for livestock. Efforts at finding cheap sources of proteins for both human and livestock are in progress and yielding good results (Sauvaitre and Baccou, 1976; Makinde *et al*, 1985; Afolabi *et al*, 1985; Udayasekhara and Sharma, 1987).

T. danielli fruit, commonly known as the Miraculous fruit of Sudan, and locally known as "katemfe" or "Ewe eran" is found throughout the West Africa rain forest zone (Dalziel, 1937). The plant grows mostly in the cocoa-growing areas of South West Nigeria, where its broad leaves are used for wrapping food, and its petioles for making mats. The fruit contains one to three hard black seeds. Each capped with a soft membranous sac called the aril and surrounded by a thin layer of transparent gel. The aril is the source of an intensely sweet protein called thaumatin and it represents 0.1% of the total fruit weight, while the remaining 99.9%, which include the pericarp and seeds, are regarded as waste (Higginbotham, 1979).

*Corresponding Author

The increasing interest in the utilization of the sweet protein (Higginbotham, 1983; Salminen and Kallinkainen, 1990) should be equally followed by interest in how the waste generated could be utilized. Adesina and Higginbotham (1977) have reported studies on the polysaccharide gel surrounding the seed. The chemical composition and some protease inhibitors in the seed and pericarp have also been studied (Elemo *et al*, 2000), and crude protein values of 4% and 10% were reported for the pulp and seed respectively.

This study was carried out to determine the levels of some antinutrients and assess the in vitro protein digestibility of *T. danielli* pulp and seed as a means of establishing its possible use in livestock formulation.

MATERIALS AND METHODS

Sample Preparation

Thaumatococcus danielli fruit were obtained at Ado Odo, Ogun State. The aril and gel surrounding the seed were removed. The pericarp and seed were oven-dried at 60°C and ground separately into fine flour with a Phillips blender. Samples were stored in airtight container for analysis. Pigs' intestinal fluid was obtained at the Lagos State Abattoir, Agege.

Antinutritive Factors Determinations

All analyses were carried out on triplicate samples of pulp and seed. Phytic acid was extracted from 1g of each sample with 0.5M Nitric acid and estimated colorimetrically according to the method described by Davies *et al* (1979). Phytic acid was expressed as nig per gram of sample. Tannins were extracted with 1% HCl in methanol

and determined by the Vanillin-HCl method of Burns (1971). Tannin content was expressed as mg D-Catechin per gram sample. Polyphenols were extracted with methanol and estimated according to the Vanillin-HCl method of Gupta and Hasain (1979). Polyphenols in *T. danielli* was expressed as mg tannic acid per gram sample. Saponins were extracted from 1g samples with 1M H_2SO_4 in dioxane water and estimation carried out colorimetrically on a spectrophotometer (Spectronic 20) according to the method of Gestetner *et al*, (1966) and expressed as mg saponin per gram sample.

Preparation of Defatted Sample

10g each of *T. danielli* pulp and seed flour was defatted with Chloroform-Methanol (2:1 v/v) mixture for 6 hours in a soxhlet extraction apparatus. The defatted flour was dried to constant weight at 50°C, and cooled in a desiccator.

Protein** solubility **test

Preliminary studies to ascertain the most efficient solvents for T. danielli protein extraction were carried out according to the method of Strength (1970). 1 g each of the defatted flour was added to 10cm^3 each of 0.05M, 1.0M and 1.5M NaCl, distilled water and I M HCl separately. After 2 hours the mixture were centrifuged at 1000g for 10mins.

Supernatants were collected for soluble protein estimation. Total nitrogen content of supernatants was determined by a modified microkjeldhal method of Fawcett and Scott (1960) as described by Chaney and Marbach (1962).

Sequential Protein Extraction

Sequential protein extraction of the defatted flour from the pulp and seed was carried out using the Osborne (1924) method described by Sodex and Wilson (1971). 4g of defatted samples were extracted three times with 20cm^3 distilled water at 4°C for 30mins each. The extracts were centrifuged at 1000g and the supernatant pooled. This represented the albumins fraction. The residue was treated in the same pattern for globulins (3 x 20cm^3 of 0.5M NaCl at 20°C), prolamins (3 x 20cm^3 of 0.2% aqueous ethanol at 20°C) and glutelins (3 x 20cm^3 of 0.2% NaOH at 20°C). Residues were washed with distilled water after each extraction procedure. The supernatants were then treated with trichloroacetic acid (5% for alburnins and 20% for other fractions), to precipitate the proteins. The precipitates were filtered and dried in a desiccator overnight. Dried samples were weighed.

In Vitro Digestibility of Defatted T. danielli Samples

The protein digestibility of defatted *T. danielli* pericarp and seed flour was assayed using the *in vitro* method described by Furuya *et al* (1979). 0.5g samples of the defatted seeds and pulp were placed in 3 different 100cm^3 Erlenmeyer flasks. 20mg of pepsin (EC 3.4.4.2) in 10cm^3 of 0.075M HCl was added and the mixture was incubated for 4 hours at 37°C. After neutralization with 0.2M NaOH. 10cm^3 of pig's intestinal fluid was added and incubated for an additional 4 hours at 37°C. After the two-stage incubation, the contents of the flasks were centrifuged for l0mins. at 1250 x g and the residue was transferred to a pre-weighed filter paper and oven-dried for dry matter and crude protein (N x 6.25) determinations. Total nitrogen was determined according to the modified microkjeldhal method described by Chaney and Marbach (1962). The *in vitro* crude protein digestibility was calculated on the basis of original crude protein contents of the defatted pulp and seed flour.

RESULTS AND DISCUSSION

Levels of antinutrients in *T. danielli* waste are generally high (Table 1), and this may limit the availability of nutrients present in it. Tannins have been claimed to adversely affect protein digestibility (Sathe and Salsunkhe, 1984), but the minimum level of tannin required to elicit a negative growth response has not been established and it is still unclear what level of tannin would be noticeably harmful. The level of tannins in the seed of *T. danielli* was twice the amount in the pericarp (Table 1) but both amount were still very low when compared to the tannin content of most sorghum grains (28-43mg/g).

T. danielli had a high content of phytate compared to most cereals (5-18mg/g) and most legumes 94-21mg/g); the level is however lower than what is found in rapeseed meal (30-50mg/g) (Robinson, 1987). The high content of phytic acid is of nutritional significance as it not only makes phytate phosphorous unavailable, but also lower the availability of essential minerals like magnesium, calcium, zinc and iron in monogastric animals and inhibits amylase activity (Reddy *et al*, 1982). However, phytate acid could be substantially eliminated by such processing methods as soaking and/or cooking (Deka and Sarkar, 1990). The level of polyphenols present in *T. danielli* pericarp was far greater than that of the seed (Table 1). Both values obtained were high but could also be eliminated by soaking and cooking. High content of dietary polyphenols like tannins diminish the permeability of the gut wall by reacting with the outer cellular layer of the gut (Ravindra and Manohar, 1984).

Dietary saponins have been reported to lower plasma and liver cholesterol concentration, and increase the excretion of bile acids and neutral sterols in feces (Oakenfull, *et al*, 1979). High content of dietary saponins however causes abdominal pain, vomiting and diarrhoea. The level of saponins in *T. danielli* waste is very low compared to 6mglg reported for soybean (Robinson, 1987).

The solubility profile showed dilute alkaline solutions to be among the best solvents for the extractions of *T. danielli* proteins. This suggests that the protein is presumably high in acidic amino acids. Increasing the molarity of NaOH from 0.05M to 1.0M led to a decrease in the extraction rate of the pericarp protein, while a marked increase in protein extract was obtained for the seed. Similar observation was obtained with increasing NaCl

concentration (Table 2).

Table 1. Levels of antinutritive factors in *Thaumatococcus danielli* pericarp and seed.

	Tannin (mg/g)	Phytic acid (mg/g)	Polyphenols (mg/g)	Saponin (mg/g)
Pericarp	4.10 ± 0.14	22.67 ± 0.47	224.17 ± 2.21	0.63 ± 0.04
Seed	7.50 ± 0.14	25.67 ± 1.03	61.67 ± 4.71	0.74 ± 0.05

Mean ± SD for triplicate samples

TABLE 2. Protein solubility profile of T. danielli pericarp and seed

	% Protein Extracted	
Solvent	**Pericarp**	**Seed**
0.5M NaCl	3.30	1.56
1.0M NaCl	2.67	3.19
1.5M NaCl	1.98	3.30
0.05MNaOH	4.51	1.35
0.10M NaOH	3.13	2.95
0.25M NaOH	2.0	3.44
0.5M NaOH	2.08	2.77
1.0M NaOH	1.80	4.86
1.0M HCl	2.77	2.29
Distilled water	3.99	3.47

TABLE 3. Solubility Classification of T. danielli waste protein

	Type of Protein	% Protein Extracted	%Protein Relative To Total Protein
Pericarp	Albumin	3.00	1090
	Globulin	9.75	35.50
	Prolamin	3.75	13.60
	Glutelin	11.00	40.00
	Total	**27.50**	**100.00**
Seed	Albumin	2.25	7.6')
	Globulin	7.50	25.64
	Prolamin	6.25	21.37
	Glutelin	13.25	45.30
	Total	**29.25**	**10000**

The sequential extraction of the pulp and seed protein indicated that glutelins constitute the major component of extractable protein of *T. danielli*, representing 40% and 45% respectively (Table 3). The high glutelin content is in line with the result of the solubility experiment and could also be attributed to the protein being rich in acidic amino acids (Makinde *et al*, 1982). This was followed by globulins and prolamins fractions. Albumins were the least extracted.

The results of *in vitro* protein digestibility of *T. danielli* pulp and seed (Table 4) showed that protein digestibility of the pulp was only slightly higher than that of the seed. The value obtained for both portions are low compared to 55% reported for palm kernel meal 30 using pepsin and pig's intestinal fluid (Balogun, 1982). The low protein digestibility of *T. danielli* waste could be attributed to the presence of antinutritive factors in the waste and the high fibre content (22% and 21% for seed and pulp respectively) (Elemo *et al*, 1999). Antinutritional factors such as trypsin inhibitors, phytate, and polyphenols have been reported to adversely affect protein digestibility (Tan *et al*, 1984; Knuches *et al*, 1985).

Resistance of glubulins to proteolytic enzymes may also be factor responsible for low protein digestibility. This has been shown by Walker and Kochhar (1983) to be the case for legumes.

It is however expected, that, processing methods like soaking and cooking would appreciably increase the protein digestibility of *T.*

danielli waste. Being a good source of protein, the waste will be useful in the formulation of livestock feed, hut it maybe necessary to carry out feeding and toxicological tests on experimental animals to establish safety and digestibility.

REFERENCES

Afolabi, O. A., Oshuntogun, B. A., Adewusi, S. R., Fagojuwo, O. O., Ayorinde, F. O. and Oke, O. I. (1985). Preliminary nutritional and chemical evaluation of raw seed from *Mucuna solanei*. *Agric. Food Chem.* 33:12.

Adesina, S. K., and Higginbotham, J. D. (1977) Studies on a novel polysaccharide gel from the fruit of *Thaumatococcus danielli* (Berth) *Carboh. Res.* 517-24.

Balogun, O. O. (1982). Extraction of nitrogen from palm kernel meal, and evaluation of digestibility of protein isolate from the meal by the *in vitro* method. *J. Agric. Food Chem.* 30:1163-66.

Burns, R. E. (1971) Winged bean tannin level, phytate content and haemagglutinating activity. *J. Agric. Food Chem.* 31:916-17.

Chaney, A. L., and Marbach, E. P. (1962). Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130-8.

Dalziel, J. M. (1937). The useful plants of West Tropical Africa. The crown agent for the colonies, Westminster, London, p. 476.

Davies, N. T. and Rend, H. (1979). An evaluation of the phytate, Zn, Cu, Fe, Mn content and Zn availability from Soya bean textured vegetable protein meat substitute or meat extenders. *Br. J. Nutri.* 41:579-619.

Deka, R. K. and Sarkar, C. R. (1990). Nutrient Composition and antinutritional factors of *Dolichos lablab* seed. *Food Chem.* 38:239-46.

Elemo, B. O., Adu, O. B. and Olayeye, O. I. (2000). Chemical Evaluation of Waste from *Thaumatococcus danielli* processing. Plant Foods for human Nutrition. 00:1-8

Elemo, B. O., Ogolo, O. C. Adeyemi, O. and Elemo G. N. (2000). Alpha amylase inhibitors from *Thaumatococcus danielli* (Benth). *Nig. J. Biochem Mol Biol. 15:161-165*

Fawcett, J. K. and Scott, J. E. (1960). A rapid and precise method for determination of urea. *J. Clin. Pathol* 31: 149-50.

Furuya, S., Sakanioto, K. and Takahashi, S. (1979). A new *in vitro* method for the estimation of digestibility using the intestinal fluid of pig. *Brit.J.Nutr* 41: 511-20.

Gestetner, B., Birk, Y., Bondi, A. and Tencer, Y. (1966). A method for the determination of sapogenin and saponin contents in soya bean. *Phytochemistry* 5:805.

Grupta, Y. P. and Hasain, M. (1979). Tannin Structure and biosynthesis. In: *Polyphenols in Cereals and Legumes*. J. H. Hulse (Ed.), IDRC, Canada, pp. 21-24.

Grupta, Y. P. (1987). Antinutritional and toxic factors in food legumes: A review. *Plant Food for Human Nutrition* 31:201-28.

Higginbotham, J. D. (1979). Protein Sweeteners. In: Dev. In Sweeteners – 1. C. A., M. Hough, K. J. Parker and A. J. Vlitos (Eds.) Applied Science Publishers, London pp. 85-123.

Higginbotham, J. D. (1983). Recent Development in non-nutritive sweeteners. In: Dev. In Sweeteners - 2 G. Grenby, T. H. Parker and M. G. Lindley (eds) Applied Science. Publishers, London, pp. 136-145.

Knuches, B. E., Zunzmicky, D. D. and Batachart, A. A. (1985). Effect of Phytate and partially hydrolyzed phytate on *in vitro* protein digestibility *J. Food Sci.* 50:1080-2.

Makinde, M. A., Egungwu, A. L., Okochi, V. I. (1982). Studies on Mango fruit endosperm: chemical composition and potential utilization. Food Ind. and Environ. Int. Symp., Budapest, Hungary.

Makinde, M. A., Elemo, B. O., Arukwe, U. and Pellet, P. (1985). Ukwa Seed (*Treculia africana*) Protein - 1. Chemical Evaluation and the protein quality. *J. Agric. Food Chem.* 33:70-2.

N.A.S. (1978). Post Harvest Food Losses in Developing countries National Academy of Sciences, Washington, D. C. pp. 7-8.

Oakenfull, D. C., Fenwick, F. D. and Hood, R. L. (1979). Effects of saponins on bile acids and plasma lipids in the rat. *Brit. J. Nutr.* 42:209-16.

Osborne, T. B. (1924). The Vegetable Proteins 2nd ed. Longmans Green and Co. London.

Ravindra, K. and Manohar, S. (1984). Tannins: their role in ruminant nutrition. *J. Agric. Food Chem.* 32:447-53.

Reddy, N. R., Sathe, S. K. and Salsunkhe, D.K. (1982). Phytates in legumes and cereals. *Adv. Food Res.* 28:1.

Robinson, D. S. (1987). Food Biochemistry and Nutritional Value. Longman Corp. U. K. Ltd. Essex pp. 33-48.

Salminen, S. and Kallinkainen, A. (1990). Sweeteners In Food Additives. A.L. Branen, P. M. Davidson and S. Salminen. (Eds.). Marcel Dekkar Inc. New York pp. 310-311.

Sathe, S. K. and Salsunkhe, D. K. (1984). Technology for the removal of unwanted components of dry bean. *CRC Crti Rev Food Sci Nutr.* 21:263.

Sauvaire, Y. and Baccou, J. C. (1976). Nutritional value of the proteins of leguminous seed. Fenugreek (*Trigonella foenum-grecum*). *Nutr. Rep. Int.* 14:527-35.

Sodex, L. and Wilson, C. (1971). Amino acid of protein isolated from normal, opaque Z and floury Z corn endosperms by a modified procedure. *J. Agric. Food Chem.* 19:1141-51.

Strength, D. R. (1970). Preparation, characterization and evaluation of coconut proteins. In: *Third Int Congress of Food Science and Technology*. P. 272.

Tan, N. H., Wong, C. and de Lumen, B. O. (1984). Relationship of tannin levels and trypsin inhibitor activity with *in vitro* protein digestibility of raw and heat treated winged bean. *J. Agric. Food Chem.* 32:819-22.

Udayasekhara Rao, P., and Sharma, R. D. (1987). An Evaluation of protein quality of fenugreek seed (*Trigonetta foenum-graecum*) and their supplementary effects. *Food Chem.* 24:1-9.

Walker, A. F. and Kochhar, N. (1983). Effect of processing, including domestic cooking on nutritional quality of legumes. *Proc. Nutr. Soc.* 21:41-8.