The effect of thermal processing on protein quality and free amino acid profile of *Terminalia catappa* (Indian Almond) seed

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Abstract The study examined the effect of various processing methods- boiling, drying and roasting- on the in vitro and in vivo protein digestibility and free amino acid profiles of *Terminalia catappa* seed. Moisture and crude protein of the various samples were determined. In vitro protein digestibility was determined after pepsin digestion. For the in vivo experiment, defatted *T. catappa* based diet was fed to 3 weeks old Wistar rats for 4 weeks and compared with animals maintained on casein based and nitrogen- free diets. The biological value (BV), net protein utilisation (NPU) and protein efficiency ratio (PER) of the diets were determined. Free amino acid composition was carried out using thin layer chromatography. Moisture was highest in the boiled *T. catappa* seed (8.30 ± 0.00 %). The raw, roasted and dried seeds had 5.55 ± 0.07 , 3.88 ± 0.22 and 3.75 ± 0.07 % respectively. Crude protein was 19.19, 18.89, 17.62 and 16.36 % in the dried, roasted, boiled and raw seeds respectively. Roasted *T. catappa* seed had the highest in vitro protein digestibility with 37.52 %, while the dried, boiled and raw samples had digestibility values of 27.57, 27.07 and 24.45 % respectively. All nine essential amino acids were present in *T. catappa* in high concentrations except methionine and tryptophan. Glutamate was present in the highest concentration. Also, free amino acids were higher in the processed seeds compared to the raw seed. Animals fed *T. catappa* diet compared favourably with the casein group, thus indicating that the protein is of good quality.

Keywords *Terminalia catappa* · Thermal processing · Protein quality · Amino acid

Introduction

The increasing challenge of feeding the growing population in developing countries has persisted for several decades. The incidence of both population explosion and urbanization has led to gross unavailability and low consumption of protein foods. Reports have shown that nutritional problems arising from inadequate protein intake will remain if efforts are not made towards other available and cheaper sources of protein (Makinde et al. 1985). Protein and micronutrient deficiencies particularly in the diets of young children and other vulnerable groups are one of the major problems facing developing countries (Latham 1997).

Oilseeds are good sources of macro and micronutrients. However, they have not been fully utilized for the nutritive potentials of their products. There is therefore a need to promote the production, processing, preparation and consumption of the oilseeds in the food-based approach to combating malnutrition (Achinewu 1998; Friedman 1996).

Terminalia catappa (Indian almond) is a deciduous tree found in the tropics which belong to the family combrataceae (Loveless 1983). The tree is often grown for shade, ornament, or for its nuts. The nut is enclosed in a fibrous flesh which is difficult to crack on account of its hard shell; which probably accounts for its low utilization (Thomson and Evans, 2006). The kernel is appreciated as a dessert nut in Asia and the Pacific. The seed of *T. catappa* contains 35–59 % of a pale oil which is very similar to true almond (*Prunus amygdalus*) oil, but is less prone to turn rancid (Howes 1948; Adu et al. 2013). Previous studies have indicated the seed as a rich source of protein, containing 17–26 % crude protein (Adewole and Olowookere 1986; Adu et al. 2004; Omeje et al. 2008).

This study was therefore intended to evaluate the nutritive quality of *T. catappa* seed protein and determine the effect of preparation methods- boiling, drying and roasting- on the amino acid composition and protein digestibility.

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Materials and methods

Preparation of sample

Terminalia catappa fruits were picked from trees growing around Surulere area of Lagos State. The pulp was manually peeled and the remains dried in a solar dryer for 48 h. The dried shell was cracked with the aid of a vice and the seed obtained. A portion of the seed was then milled into powder with a Philips blender after drying in an oven between 150 and 200 °C for 2 h. The milled sample was autoclaved for 30 min at 120 °C to inactivate the antinutrients. This sample was used to compound the *T. catappa* based diet. Other portions of the dried seed were treated and labeled as follows:

- Boiled: The seeds were boiled in water at 100 °C for 10 min and drained
- Dried: The seeds were dried in a solar dryer for 24 h
- Roasted: The seeds were placed in an oven and roasted at 50–100 °C for 10 min
- Raw: These were left unprocessed

The various samples were milled in a blender, placed in air-tight plastic containers and kept in the refrigerator until needed. These samples were used for moisture and crude protein determination, as well as in vitro digestibility experiments.

In vitro protein digestibility

One gram of milled *T. catappa* samples was weighed into a 500 ml Erlenmeyer flask and 150 ml of prewarmed solution of pepsin (0.2 % w/v) in 0.075 N HCl was added with gentle stirring. The sample was digested for 16 h at 42–45 °C in a shaker incubator. The digested sample was then dialysed for 24 h against Tris HCl buffer (pH 8.0). The dialysate was filtered through a buchner funnel attached to a vacuum pump and washed twice with 15 ml of acetone. The residue was later dried at 105 °C for 4 h and weighed. Crude protein was determined in both undigested and digested samples and the percentage protein digestibility was calculated as:

$$\% \text{Protein digestibility} = \frac{CP_u - CP_d}{CP_u} \times 100$$

Where, CP_u —crude protein of undigested sample and CP_d —crude protein of digested sample

In vivo protein digestibility

Three weeks old albino rats (*Rattus norvegicus*) (Wistar strain) were obtained from the animal house of the Department of Pharmacology, University of Jos, Plateau State. The animals were placed in groups of three in 3

metabolic cages and allowed to stabilize for 4 days. On the fifth day, the animals were starved over night, after which they were placed on the formulated diets (Table 1) for 4 weeks. Group 1 was assigned protein-free diet; Group 2, casein based diet and Group 3 *T. catappa* based diet. The animals were allowed free access to food and water, and daily records of food intake, faecal and urine output, weight gain and room temperature were taken.

Free amino acid determination

Two grams of raw and treated *T. catappa* samples were weighed into a 250 ml beaker and 20 ml of 0.2 M phosphate buffer (pH 7.0) was added. The mixture was stirred for 5 min and the centrifuged at 2,000 rpm in a Haereus Sepatech centrifuge for 10 min. The supernatant was then transferred into a separating funnel and washed 3 times with 10 ml of petroleum ether to remove the organic pigments. The aqueous phase was retained. Protein was precipitated by adding 5 ml of 10 % trichloroacetic acid (TCA) to 5 ml of the aqueous extract. The mixture was shaken and kept at −10 °C for 10 min. The precipitate was removed by centrifugation at 2,000 rpm for 10 min and the supernatant was used for amino acid determination.

Thin layer chromatography

Amino acids were separated by TLC on an Avicel microcrystalline cellulose plate. One-dimensional ascending chromatography was carried out; and the solvent system was n-butanol-acetic acid-water (4: 1: 2).

Quantitative determination of free amino acid

Free amino acid concentration was determined by the use of the guided strip technique. Squares containing amino acids were cut out and eluted with 5 ml distilled water at 70 °C for 2 h. The cellulose powder was then removed by centrifugation

Table 1 Composition of diet (g)

Component	Casein-based diet	<i>T. catappa</i> diet	Protein-free diet
Corn starch	430	780	430
Casein	4	—	—
<i>T. catappa</i>	—	350	—
Soybean oil	40	40	40
Sucrose	100	100	100
Cellulose (rice husk)	40	40	40
Vitamin mix	10	10	10
Mineral mix	40	40	40
DL- Methionine	4	4	4

at 200 rpm for 20 min. Amino acid concentration was determined by the modified ninhydrin method (Rosen, 1957).

Analyses

Moisture content was determined by the standard AOAC (1990) method. Nitrogen was determined according to the micro Kjeldhal method (AOAC 1990). Crude protein was calculated by multiplying the nitrogen value by a factor of 6.25.

Results

The moisture, crude protein content and protein digestibility of *T. catappa* seed are shown in Table 2. Moisture content was highest in boiled *T. catappa* seed (8.30 ± 0.00 %). This value was significantly higher ($p < 0.05$) than those of raw (5.55 ± 0.07 %), roasted (3.88 ± 0.22 %) and dried seeds (3.75 ± 0.07 %). Dried and roasted *T. catappa* seeds had significantly higher crude protein content (19.19 ± 1.35 % and 18.89 ± 0.70 % respectively) compared to boiled and raw seeds (17.62 ± 0.85 and 16.36 ± 1.23 % respectively). The protein digestibility of *T. catappa* nut ranged from 24.45 ± 1.79 % to 37.52 ± 2.66 % in the order: raw < boiled < dried < roasted. Protein digestibility of roasted *T. catappa* seed was however significantly higher ($p < 0.05$) than those of other samples. The raw seed had significantly lower crude protein and protein digestibility compared to the thermal processed seeds.

The mean weekly feed intake and weight gain by the experimental animals are shown in Table 3. There was no difference in feed intake by both casein-based and *T. catappa*-based groups. Both groups however had significantly higher feed intake ($p < 0.05$) compared to the protein free diet. Weight gain was significantly higher ($p < 0.05$) in the animals placed on the casein-based diet up until the third week. By the fourth week, however, the difference in weight between the casein-based and *T. catappa* groups was no longer significant.

Table 2 Moisture, crude protein and in vitro digestibility of *T. catappa* seed*

Sample	Moisture (%)	Crude protein (%)	Protein digestibility (%)
Raw	5.55 ± 0.07^b	16.36 ± 1.23^a	24.45 ± 1.79^a
Boiled	8.30 ± 0.00^c	17.62 ± 0.85^b	27.07 ± 1.25^b
Dried	3.75 ± 0.07^a	19.19 ± 1.35^c	27.57 ± 0.90^b
Roasted	3.88 ± 0.22^a	18.89 ± 0.70^c	37.52 ± 2.66^c

Values are mean $\pm$ Standard error of triplicate sample determinations

* Values with different superscript within columns are significantly different at $p < 0.05$

Table 3 Feed intake and weight gain by animals fed *T. catappa* meal

Diet group	Week	Feed intake (g)	Weight gain (g)
Protein-free diet	1	12.19 ± 0.05^a	1.86 ± 0.33^a
	2	12.30 ± 0.06^a	1.89 ± 0.02^a
	3	12.26 ± 0.09^a	1.92 ± 0.03^a
	4	10.34 ± 4.53^a	1.83 ± 0.13^a
	Total	11.77 ± 2.30	1.87 ± 0.17
Casein-based diet	1	17.86 ± 0.63^b	3.51 ± 0.33^c
	2	17.50 ± 0.08^b	3.94 ± 0.04^c
	3	17.80 ± 0.32^b	3.78 ± 0.17^c
	4	17.30 ± 0.12^b	3.67 ± 0.04^{bc}
	Total	$17.62 \pm 0.41^\dagger$	$3.73 \pm 0.24^\dagger$
<i>T. catappa</i> diet	1	17.09 ± 0.12^b	3.30 ± 0.21^b
	2	17.11 ± 0.31^b	3.58 ± 0.37^b
	3	17.45 ± 0.09^b	3.43 ± 0.03^b
	4	17.55 ± 0.11^b	3.52 ± 0.05^{bc}
	Total	$17.30 \pm 0.27^\dagger$	$3.46 \pm 0.23^{\dagger\ddagger}$

Values are mean $\pm$ Standard error of triplicate sample determinations

* Values with different superscript across columns are significantly different at $p < 0.05$

† Value is significantly different from protein-free diet at $p < 0.05$

‡ Values are significantly different from casein-based diet at $p < 0.05$

In vivo evaluation of the *T. catappa* meal compared to casein diet (Table 4) showed that nitrogen balance (NB), biological value (BV), and protein efficiency ratio (PER) of the *T. catappa* diet (2.01, 30.80 and 0.18 respectively) were similar to those of the casein diet (2.89, 31.85 and 0.16 respectively).

Fifteen amino acids were detected in all the *T. catappa* samples (Table 5). All nine essential amino acids were present but at low concentrations. Glutamate was present in the highest concentration (22.42 – 23.50 g/100 g) and tryptophan was the least (0.16 – 0.32 g/100 g). The processed seeds had relatively higher free amino acids concentration than the raw sample (Table 5).

Table 4 Biological evaluation of *T. catappa* seed

Protein quality	Protein-free diet	Casein-based diet	<i>T. catappa</i> diet
Nitrogen balance	0.13	2.89	2.01
Biological value (%)	−182.16	31.85	30.8
Protein efficiency ratio	0.33	0.16	0.18
Faecal nitrogen	0.58	0.42	0.44
Urine nitrogen	0.63	0.59	0.66
Nitrogen utilized	0.24	0.92	0.62
True nitrogen absorbed	−0.13	2.89	2.14
Protein intake	5.78	24.39	19.51

Table 5 Free amino acid content of *T. catappa* nut

Amino Acid	Amino acid concentration (g/100 g)			
	Raw	Boiled	Dried	Roasted
Arginine	2.68	2.37	3.53	3.37
Histidine	0.63	0.74	1.16	1.05
Isoleucine	0.84	0.89	1.16	1.05
Luecine	1.26	1.42	1.53	1.42
Lysine	0.68	0.79	1.00	0.89
Methionine	0.26	0.26	0.42	0.47
Phenylalanine	1.00	1.11	1.37	1.16
Threonine	0.74	0.84	0.89	0.79
Tyrosine	0.63	0.68	0.78	0.74
Tryptophan	0.16	0.21	0.32	0.26
Valine	0.79	0.79	0.95	1.00
Cysteine	0.89	1.00	1.10	1.21
Glycine	0.58	0.68	0.74	0.84
Glutamic acid	22.42	22.79	23.50	23.00
Total	33.56	34.57	38.45	37.25

Discussion

The flesh of the Indian almond is known to be more consumed compared to the nuts and during the fruiting season, so much of the fruit gets wasted, representing a substantial loss of nutrient. Roasted nuts, without any additives or with spices, have become popular snacks. They constitute a valuable raw material in the industries like confectionery, bakery and others. Most often roasted are such nuts like peanuts, hazelnuts, almonds and pistachio nuts (Kita and Figiel 2007).

The moisture content of food is usually an index of its stability and quality. It is also a measure of its yield and quantity of food solids (FIRO 1996). The moisture content of raw *T. catappa* nut was generally low and it was comparable to those of almond (3.6–4.4 %), raw cashew nut (6 %) and peanut (Aslantas et al. 2001). Drying and roasting significantly reduced the moisture content of the nut. For the prevention of aflatoxin during storage, a maximum of 10 % moisture content is recommended as safe moisture level for peanut and 6.5 % has been recommended for almond (UNECE 2003).

Terminalia catappa is a good source of protein with some reports indicating values as high as 43 % in the defatted seed (Oderinde and Ajayi 1998). In this study, the raw seed contained 16.36 % crude protein. This seemingly low value obtained might be due to the fact that the seed was not defatted. Thermal processing of the seed improved its crude protein content. Drying, roasting and boiling (in descending order) significantly improved ($p < 0.05$) the availability of protein. Adegunwa et al. (2012) reported no significant effect of thermal processing (cooking, roasting and autoclaving) on the protein content of beniseed. However, Abdulsalami and

Sheriff (2010) reported substantial increase in crude protein after soaking and cooking of bambara groundnut (*Voandzeia subterranean*). This increase was attributed to the destruction of antinutritional factors resulting in release of nutrients. In this study, processing methods that reduced moisture content had greater effect on improving protein content. Dehydration as a food processing method is known to increase nutrient density of most foods.

Although the biological availability of protein in foods must, in the final analysis, be established by exact feeding trials, in vitro methods of evaluating protein digestibility are important because they are rapid and sensitive. They are particularly useful where foods are being processed in a large number of ways, and where animal feeding trials would be tedious, expensive, and incapable of detecting small differences (Saunders and Kohler 1972). Digestibility of *T. catappa* protein was relatively low, but roasting of the nut substantially improved protein digestibility by as much as 53 %. Boiling and drying also significantly improved protein digestibility but only by 11–13 %. This indicates that dry heating (roasting) had more beneficial effect on digestibility of *T. catappa* protein than wet heating (boiling). This was however contrary to the report of Pugalenti et al. (2007), that wet heating of *Abrus precatorius* seeds significantly improved its protein utilization. Improvement in the protein quality after thermal treatment might be attributed to reduction on the levels of various antinutrients in addition to some other factors such as disruption of protein structure and increased accessibility of the seed proteins to enzymatic attack (Nielson 1991). Dehydration however only increased nutrient density but did not substantially improve digestibility. Previous comparison of the biological value of raw *T. catappa* with that of soy protein showed that the protein was poorly digested and utilized. *Aspergillus niger* fermentation however improved its performance significantly (Muhammad and Oloyede 2004; Muhammad and Oloyede 2010).

Apart from the fact that free amino acids readily contribute to the nutrient need of the body, they contribute to taste, aroma and colour development of foods (Kirimura et al. 1969; Fabiani et al. 2002). They are precursors for the production of the key flavour compounds (Janiszewska et al. 2012). Nine of the fifteen amino acids detected in *T. catappa* nut were essential amino acids. Amino acid concentrations were relatively high except methionine and tryptophan. Drying and roasting of the nut increased its free amino acid profile. It is important to note that asparagine was not detected in *T. catappa* nut. Asparagine is the main free amino acid in raw almonds (*P. amygdalus*) and is correlated with the acrylamide content of dark roasted almonds. Acrylamide is a known carcinogen (Rice 2005). Glutamate was present in the highest concentration. This was similar to the report of Muhammad and Oloyede (2009), but the values obtained from our study was higher.

Conclusion

Raw *T. catappa* nut is an underutilized food source that is rich in protein but low on protein digestibility. Roasting of the nut significantly increased the crude protein content and protein digestibility in vitro. On the other hand, drying and boiling of the nut only increased the protein content, but did not substantially increase its digestibility. In vivo protein quality assessment of the roasted nut suggests a protein that is comparable to casein. Fifteen amino acids were also detected in the nut out of which nine were essential amino acids. These amino acids were present in high concentrations except methionine and tryptophan.

References

- Abdulsalami MS, Sheriff HB (2010) Effect of processing on the proximate composition and mineral content of bambara groundnut (*Voandzeia subterranean*). J Pure Appl Sci 3(1):188–190
- Achinewu SC (1998) Nuts and oil seeds. In: Osagie AU, Eka OU (eds) Nutritional quality of plant foods, post harvest research unit. UNIBEN, Benincity, pp 134–159
- Adegunwa MO, Adebawale AA, Solano EO (2012) Effect of thermal processing on the biochemical composition, antinutritional factors and functional properties of beniseed (*Sesamum indicum*) flour. Am J Biochem Mol Biol 2(3):175–182
- Adewole A, Olowookere JO (1986) Nutritional potentialities of *Irvingia gabonensis* and *Terminalia catappa* fruits. Nutr Repts Int 2:10–13
- Adu OB, Sadiq OTI, Ajiboro OO, Nnaife CM, Ogunrinola OO, Elemo BO (2004) Proximate composition and mineral bioavailability of indian almond (*Terminalia catappa*) seed. Proceedings, 28th annual conference, Nigerian institute of food science and technology, Ibadan, Pp 129–130.
- Adu OB, Omojufehinsi M, Esanboro MO, Abe DA, Shofolahan AO, Uzodinma E, Badmus K, Martins O (2013) Effect of processing on the quality, composition and antioxidant properties of *Terminalia catappa* (indian almond) seed oil. Afr J Food Agric Nutr Dev 13(3): 7662–7678
- AOAC (1990) Association of official analytical chemists. Official methods of analysis, 15th Edn., Washington DC.
- Aslantas R, Guleryuz M, Turan M (2001) Some chemical contents of selected almond (*Prunus amygdalus* Batsch) types. In: Ak B.E. (ed.) XI GREMPA seminar on pistachios and almonds. Zaragoza: CIHEAM, 2001, p.347–350 (Cahiers Options Mediterraneennes: n56)
- Fabiani A, Versari A, Parpinello GP, Castellari M, Galassi S (2002) High-performance liquid chromatographic analysis of free amino acids in fruit juices using derivatization with 9-fluorenylmethyl-chloroformate. Journal of Chromatographic Science, Vol. 40, January 2002
- FIIRO (1996) Technical papers, federal institute of industrial research. Oshodi, Lagos
- Friedman M (1996) Nutritional value of proteins from different food sources. a review. J Agric Food Chem 44:6–29
- Howes FN (1948) *Nuts*. Their production and everyday use. Faber and Faber, London, pp 209–220
- Janiszewska K, Aniolsowska M, Nowakowski P (2012) Free amino acids content of honeys from Poland. Pol J Food Nutr Sci 62(2):85–89. doi:10.2478/v10222-011-0041-5
- Kirimura J, Shimizu A, Kimizuka A, Ninomiya T, Katsuya N (1969) Contribution of peptides and amino acids to the taste of foods. J Agric Food Chem 17(4):689–695. doi:10.1021/jf60164a031
- Kita A, Figiel A (2007) Effect of roasting on properties of walnuts. Pol J Food Nutr Sci 57(2(A)):89–94
- Latham MC (1997) Human nutrition in the developing world, 29th edn. FAO Food and Nutrition Series, Rome
- Loveless AR (1983) Principles of plant biology for the tropics. Longman Group Limited, Lincoln, United Kingdom, pp 407–415
- Makeinde MA, Elemo BO, Arukwe U, Pelett P (1985) Ukwa seed (*Trecularia africana*) protein: chemical evaluation of the protein quality. J Agric Food Chem 33:70–72
- Muhammad NO, Oloyede OB (2004) Assessment of biological value of *Terminalia catappa* seed meal-based diet in rats. Biokemistri 16(1): 49–55
- Muhammad NO, Oloyede OB (2009) Protein fractions and amino acid profile of *Aspergillus niger*-fermented *Terminalia catappa* seed meal. Afr J Microbiol Res 3(3):101–104
- Muhammad NO, Oloyede OB (2010) Growth performance of broiler chicks fed *Aspergillus niger*-fermented *Terminalia catappa* seed meal-based diet. Int J Biol Chem Sci 4(1):107–114
- Nielson SS (1991) Digestibility of legume proteins. Food Technol 45: 112–118
- Oderinde RA, Ajayi IA (1998) Metal and oil characteristics of *Terminalia catappa*. La Rivista Italiana Sostanze Delle Grasse. 75:361–362
- Omeje EO, Okide GB, Esimone CO, Ajali U (2008) Kinetics of autoxidation of an oil extract from *Terminalia catappa*. Indian J Pharm Sci 70:260–2, Available from: <http://www.ijpsonline.com/text.asp?2008/70/2/260/41472>
- Pugalenth M, Vadivel V, Janaki P (2007) Comparative evaluation of protein quality of raw and differentially processed seeds of an underutilized food legume, *Abrus precatorius* L. Livestock Research for Rural Development volume 19, article #11 <http://www.cipav.org.co/lrrd/lrrd19/11/cont1911.htm>
- Rice JM (2005) The carcinogenicity of acrylamide. Mutat Res 580:3–20
- Rosen A (1957) A modified ninhydrin colorimetric analysis for amino acids. Arch Biochem Biophys 67:10–15
- Saunders RM, Kohler GO (1972) In vitro determination of protein digestibility in wheat millfeeds for monogastric animals. Cereal Chem 49:98–103
- Thomson LA, Evans B (2006) *Terminalia catappa* (tropical almond). Species profiles for pacific island agroforestry. <http://www.traditionaltree.org>.
- UNECE (2003) Concerning the marketing and commercial quality control of almond kernels. UNECE Standard DDP-06. TRADE/WP.7/GE.2/2003/15/Add.1. www.unece.org/trade/agr.