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Protein fractions and amino acid profile of *Aspergillus niger*-fermented *Terminalia catappa* seed meal

Muhammad, Nasiru Olanrewaju[1]* and Oloyede, Oyelola Bukoye[2]

[1]Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.
[2]Office of the Vice-Chancellor, Fountain University, Osogbo, Nigeria.

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The effect of solid state fermentation, using *Aspergillus niger*, on the protein fractions and amino acid profile of *Terminalia catappa* seed meal was investigated. A 5 ml *A. niger* spore suspension, containing 2.21 x 10^4 spores per ml, was used to inoculate 25 g of milled *T. catappa* seed meal. The inoculated samples were then incubated at room temperature (25 – 30^{o}C) for 1, 2 or 3 weeks. Levels of albumin, globulin, gliadin and glutelin, as well as the essential and non- essential amino acids profile were determined in both the raw and the fermented samples. The results showed that glutelin and gliadin are the predominant protein fractions (74.07%) in *T. catappa* seed meal, while albumin and globulin are less prominent (25.93%). Fermentation with *A. niger*, however, significantly increased the albumin and globulin content of the *T. catappa* seed meal to 38.83%, while it significantly reduced the gliadin fraction. The glutelin fraction was, however, not significantly (p>0.05) affected by the treatment. There were significant reductions (p<0.05) in some of the essential amino acids (like leucine, isoleucine and methionine), while the non-essential amino acids like glutamic acid was significantly increased (p<0.05). It is considered that fermentation using *A. niger* could improve the nutritive protein fractions of *T. catappa* seed meal. However, there may be need for essential amino acid supplementation when such fermented seed meal is used in formulating feed for poultry and livestock animals.

Key words: *Aspergillus niger*, fermentation, *Terminalia catappa* seed, protein fractions, amino acids.

INTRODUCTION

Plant seeds are employed as sources of protein in man, livestock and poultry feeds. In plant seeds, protein frac-tions include albumin, globulin, gliadin and glutelin (Alais and Linden, 1999). Albumin is found in both plants and animal tissues. It is a nutritive material that fills the space in the seed between the embryo and the seed coat. It is also found in grains, occupying 30% of the total protein in conjuction with globulins (Tella and Ojehomon, 1980; Alais and Linden, 1999). Globulin is also present in both plants and animals. In oats and rice, globulin forms the major endosperm storage protein fraction, accounting for 70 – 80% of the total protein (Casey, 1999) . Both albumin and globulin are also readily soluble in salt solution (Alais and Linden, 1999). Gliadin, also known as prolamin, is found in most cereals. About 69% is found in wheat. They are called prolamins because they are generally rich in proline and amide nitrogen derived from glutamine. They are soluble in about 70 – 90% alcohol and occur mostly together with glutelin (Alais and Linden, 1999). Glutelin are the major proteins found in wheat, rye and barley (Alais and Linden, 1999). Glutelin is crosslinked through disulfide bonds, present in cystine residues, which render the protein insoluble in both saline and 70% ethanol solu-tions (Nielsen et al., 1968). They are, however, soluble in dilute acid or alkali solutions. Occurrence of glutelin and gliadin together is known as gluten (Alais and Linden, 1999).

Though some plants seeds and grains are rich in pro-teins, utilization of fractions thereof in poultry and live-stock animals are limited due to the presence of antinutri-tional factors (Osagie, 1998). Some antinutrients (like tannins) are known to complex with starch and protein components of foods, thereby reducing their digestibility and consequently, their bioavailability (Salunkhe et al., 1990). Tannins, for example, reported to be present in

*Corresponding author. E-mail: alphamno2@yahoo.com. or muno@unilorin.edu.ng. Tel. +234)8033931900.

Table 1. Protein fractions of *Terminalia catappa* seed meal fermented by *Aspergillus niger* for 3 weeks

Protein fraction	Raw	1week	2 weeks	3 weeks
Albumin (mg/g)	12.44 ± 0.09[a]	13.13±0.18[b]	14.69 ± 0.09[c]	15.13 ± 0.08[d]
Globulin (mg/g)	7.56 ± 0.09[a]	8.07±0.09[b]	9.25 ±0.07[c]	12.13 ± 0.18[d]
Glutelin (mg/g)	36.88 ± 0.30[a]	36.88±0.25[a]	36.88 ± 0.39[a]	36.88 ± 0.28[a]
Gliadin (mg/g)	20.25 ± 0.35[a]	10.75±0.35[b]	8.46 ± 0.19[c]	6.06 ± 0.09[d]
Total Isolatable Protein (mg/g)	77.13 ± 1.43[a]	68.85±1.55[b]	69.28 ± 1.23[c]	70.20 ± 1.35[d]
% Water-soluble fractions (Albumin & Globulin)	25.93	30.79	34.56	38.83
% Prolamine (Gliadin & Glutelin)	74.07	69.18	65.44	61.17

Values are means of 4 determinations ± SD.
Row values with different superscripts are significantly ($p<0.05$) different.

Terminalia catappa seed (Jeremiah, 1992; Muhammad and Oloyede, 2006), are known either to cross-link with dietary protein by reacting with lysine or methionine, making them unavailable during digestion (Vidal-Verlvede et al., 1994), or form complexes with digestive enzymes (Macrae et al., 1993). Other antinutritional factors like phytate, oxalate and cyanide are known to chelate with the mineral elements, especially the divalent ions reducing their bioavailability (Butler, 1989, 1992; John, 2000).

Solid state fermentation (using fungi) as a processing method has, however, been reported to increase the crude protein and inorganic minerals, and decrease the lipid, fibre and antinutrient contents of plant materials (Jacqueline et al., 1996; Muhammad et al., 2000; Belewu et al., 2006; Muhammad and Oloyede, 2006). However, there is paucity of information on the effect of this processing method on the protein fractions and levels of individual amino acids in plant food materials, thus the need for the present study.

MATERIALS AND METHODS

Processing of *Terminalia catappa*

Ripe fruits of *T. catappa* (authenticated at FRIN, Ibadan, Nigeria, with a voucher number of FHI 107767) were picked from the premises of the main campus of the University of Ilorin, Ilorin, Nigeria, and oven-dried at 60^{o} C and cracked to remove the seeds, using a 125 mm Bench vice. The seeds were then milled using a magic blender (model SHB-515, Sorex Company Limited, Seoul, Korea).

Inoculation of substrate with *Aspergillus niger*

Aspergillus niger was obtained from the Plant Health Management Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Spores of *A. niger* were sub-cultured on potato dextrose agar (PDA) that had been autoclaved at 121^{o}C and 15 kgcm^{-2} for 15 min. Colonies of the cultured fungus were suspended in sterile distilled water. A 5 ml spore suspension, containing 2.21 x 10^{4} spores of *A. niger* per ml, was used to inoculate 25 g of autoclaved *T. catappa* seed meal. This was done in an inoculation chamber (room). The inoculum was turned well with the *T. catappa* seed meal and covered with a dark polythene sheet to provide the necessary darkness for the fermentation (Belewu et al., 2002) . The samples were watered and aerated at intervals and the growth of *A. niger* was terminated after 1, 2 and 3 weeks of incubation by oven-drying the spent substrates at 60^{o}C for 48 h (to kill the *A. niger*).

Extraction and isolation of the protein fractions of fermented *T. catappa* seed meal

The method employed by Tella and Ojehomon (1980), which utilizes buffered aqueous NaCl solution to isolate albumin and globulin, dilute acid or alkaline solutions to isolate glutelin and 80% ethanol to isolate gliadin fractions, was used. The isolated fractions were subsequently quantified using the biuret reaction method (Gornall et al*.,* 1949).

Analysis of the amino acid content of fermented *Terminalia catappa* seed meal

The amino acid profile of the samples was determined using the method described by Spackman et al. (1958). The samples were dried to constant weight, defatted, hydrolysed, evaporated using a rotatory evaporator and then loaded into a Technicon Sequential Multisample Acid Analyzer (TSM).

RESULTS

Table 1 shows the effect of solid state fermentation, using *A. niger* over a period of 3 weeks, on the albumin, globulin, gliadin and glutelin contents of *T. catappa* seed meal. The reserve proteins, glutelin and gliadin, are the predominant protein fractions (74.07%) in *T. catappa* seed meal, while the water-soluble proteins, albumin and globulin, are less prominent (25.93%). The fermentation with *A. niger,* however, significantly increased the albumin and globulin content of the *T. catappa* seed meal (38.83% by the third week of fermentation), while it significantly reduced the gliadin fraction. The glutelin fraction was, however, not significantly ($p>0.05$) affected by the treatment. The reduction in the gliadin fraction eventually led to a significant decrease in the prolamine content of the fermented sample (61.17%) by the third week of fermentation. Table 2 shows the amino acid profile of both the raw and *A. niger*-fermented *T. catappa* seed meal.

Table 2. Amino Acid level (g/100g protein) of *Terminalia catappa* seed meal fermented by *Aspergillus niger* for 3 weeks

Amino acid	Raw	Weeks of Fermentation			% Inc/Dec	FAO/WHO* Ref.
		1	2	3		Standard (1973)
Lysine	6.66 ±0.02^{a}	6.05±0.04^{b}	5.75±0.015^{c}	4.26±0.042^{d}	36	4.2
Histidine	2.38 ± 0.01^{a}	2.30±0.02^{b}	2.18±0.014^{c}	1.92±0.012^{d}	19.3	2.4*
Arginine	6.52±0.013^{a}	6.13±0.03^{b}	5.82±0.021^{c}	3.88±0.051^{d}	40.5	2.0*
Aspartic acid	10.89±0.04^{a}	10.03±0.05^{b}	8.19±0.18^{c}	8.19±0.034^{c}	24.8	-
Threonine	3.02±0.012^{a}	2.99±0.02^{a}	2.29±0.11^{c}	2.01±0.014^{d}	33	2.8
Serine	4.00±0.018^{a}	3.60±0.09^{b}	3.20±0.023^{c}	2.03±0.022^{d}	49.3	-
Glutamic acid	12.07±0.02^{a}	14.21±0.20^{b}	15.12±0.05^{c}	15.84±0.03^{d}	31.2	-
Proline	3.47±0.018^{a}	3.47±0.024^{a}	3.47±0.015^{a}	3.47±0.017^{a}	0	-
Glycine	4.00±0.027^{a}	3.40±0.017^{b}	3.19±0.022^{c}	2.19±0.019^{d}	45.3	-
Alanine	1.58±0.017^{a}	3.23±0.011^{b}	3.50±0.041^{c}	4.19±0.025^{d}	165.2	-
Cysteine	1.61±0.018^{a}	1.53±0.012^{b}	1.37±0.021^{c}	1.12±0.011^{d}	30.4	2
Valine	3.62±0.019^{a}	3.49±0.022^{b}	3.49±0.012^{b}	2.01±0.013^{d}	44.5	4.2
Methionine	1.23±0.018^{a}	1.21±0.016^{a}	1.10±0.012^{c}	0.81±0.010^{d}	34.2	2.2
Isoleucine	4.51±0.026^{a}	4.35±0.019^{b}	4.21±0.015^{c}	2.05±0.022^{d}	54.5	4.2
Leucine	7.07±0.019^{a}	6.94±0.012^{b}	6.80±0.025^{c}	3.03±0.016^{d}	57.1	4.2
Tyrosine	3.18±0.015^{a}	3.04±0.013^{b}	2.49±0.020^{c}	2.21±0.044^{d}	30.5	2.8
Phenylalanine	1.75±0.014^{a}	3.86±0.023^{b}	4.06±0.035^{c}	4.14±0.019^{c}	136.6	2.8
Total	77.56±0.35	79.83±0.50	76.10±0.45	63.35±0.58		

Values are means of 4 determinations ± SD.
Row values with different superscripts are significantly (p<0.05) different.

Table 3. Changes in Essential amino acids of *Terminalia catappa* seed meal fermented by *Aspergillus niger* for 3 weeks

Amino acids	Raw	Weeks of Fermentation		
		1	2	3
Essential (g/100g)	38.37	38.85	37.07	25.23
%	49.47	49.47	48.71	39.83
Non-essential (g/100g)	39.19	40.98	39.03	38.12
%	50.53	51.33	51.29	60.17

There were significant reductions (p<0.05) in some of the essential amino acids, while the non-essential amino acids like glutamic acid was significantly increased (p<0.05). Histi-dine and proline levels in the *T. catappa* seed meal were not significantly affected (p>0.05) by the treatment.

The percentage of the essential amino acids content of the *T. catappa* seed meal was significantly (p<0.05) re-duced, while there was a significant (p<0.05) increase in the non-essential amino acids throughout the period of fermentation (Table 3). The proportion of the essential amino acids was reduced from 49.47% to 39.83% by the third week of fermentation by *A. niger,* while the non-essential amino acids increased from 50.53% to 60.17%.

DISCUSSION

The significant increase in the albumin and globulin levels of the fermented *T. catappa* seed meal (Table 1) indicates a higher nutritive value of the fermented sample, and the reduction in the gliadin content would make the fermented *T. catappa* seed meal a good source of protein in both poultry and livestock feeds. The relative proportion of each fraction in total seed protein samples had been reported to strongly affect the nutritional quality of the seed (Johnson and Lay, 1974; Sylvester-Bradley and Folkes, 1974). For instance, a positive correlation had been reportedly found between the albumin content and nutritive value in peas and other legumes (Bajaj et al., 1971a, 1971b; Bajaj, 1972), and between glutelin and biological value (Mertz et al., 1965). This had led to the idea of using measures of these protein fractions as indicators of protein quality (Tella and Ojehomon, 1980). It had equally been shown that a serious problem in the capacity to digest foods, which is called 'coeliac disease', is caused by peptides rich in glutamine, resulting from the proteolysis of gliadins (Alais and Linden, 1999). The significant increase in Ala (165.2%) and Glu (31.2%), which are the predominating amino acids in the albumin fraction (Alais and Linden, 1999), might explain the significant increase in the albumin content of the fermented seed meal as earlier observed (Table 1). Also, the significant increase in Ala (165.2%) and Phe (136.6%), which are among the predominating amino acids in globulin fraction of protein seed (Alais and Linden, 1999), might explain the significant increase in the globulin content of the seed meal. The unchanged quantity of proline of the fermented *T. catappa* seed meal, which is one of the major amino acid predominating in

glutelin fraction (Alais and Linden, 1999), might justify the constant glutelin content of the fermented seed meal throughout the period of fermentation (Table 1).

The significant reductions in the leucine (57.1%), isoleucine (54.5%), serine (49.3%) and valine (44.5%) (Table 2), the predominating amino acids in gliadin (Alais and Linden, 1999), might probably be responsible for the significant reduction in the gliadin content of the fermented seed meal (Table 1).

The significant increase in the lysine, arginine and phenylalanine content of the *A. niger*-fermented *T. catappa* seed meal (Table 2) compared favourably with WHO/FAO standards, while threonine, leucine and tyrosine are numerically close to the WHO/FAO values (FAO/WHO, 1973). The significant increase in the non-essential amino acids over the essential amino acids (Table 2) and the significant increase in the crude protein content, reported in our previous work (Muhammad and Oloyede, 2006), of the *T. catappa* seed meal throughout the period of fermentation, may be an indication that the non-essential amino acids and nucleic acids have been synthesized at the expense of the essential amino acids, thus the reduction in the essential amino acids.

Furthermore, it had been reported that some of the nutrients required for the germination of conidiospores of *A. niger* include among others, valine, leucine, cysteine and arginine (Abdel-Rahim and Arbab, 1985), thus the reduction in some of these essential amino acids (Tables 2 and 3) in the fermented *T. catappa* seed meal.

Overall, qualitatively, fermentation using *A. niger* improved the nutritive protein fractions of *T. catappa* seed meal. However, there may be a need to supplement the essential amino acids in the feed formulated with the *T. catappa* seed meal fermented by *A. niger* (as a source of protein) to ensure adequate growth of the animals consuming the feed.

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