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Processing Effects on Some Antinutritional Factors and *In vitro* Multienzyme Protein Digestibility (IVPD) of Three Tropical Seeds: Breadnut (*Artocarpus altilis*), Cashewnut (*Anacardium occidentale*) and Fluted Pumpkin (*Telfairia occidentalis*)

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Abstract: Mature seeds of breadnut, cashewnut and fluted pumpkin were processed in the laboratory into the raw dried, boiled, fermented, germinated and roasted seeds. Differently processed seeds were dried at 50°C, ground and sieved through 500μm sieves. The seed flours were evaluated for trypsin inhibitor activity, tannin, phosphorus compounds and *in vitro* multienzyme protein digestibility (IVPD). The results show that processing significantly (P = 0.05) affected the antinutritional factors in the seed flours. Breadnut flours contain 2.8-5.3g/kg phytic acid, 5.8-9.2g/kg tannin and 0.9-8.1mg/g flour of trypsin inhibitor activity. Cashewnut flours contain 6.0-9.9g/kg phytic acid, 5.1-13.3g/kg tannin and 0.8-2.5mg/g flour of trypsin inhibitor activity. Fluted pumpkin seed flours contain 2.8-13.8g/kg phytic acid, 7.5-19.1g/kg tannin and 0.0-11.0mg/g flour of trypsin inhibitor activity. Fermentation is the most effective processing method to reduce phytic acid and trypsin inhibitor activity while boiling is most effective in reducing the tannin content. The result of IVPD of the seeds generally show that the boiled samples are the most digestible followed by the fermented samples while the raw dried/germinated samples are the least. The order of digestibility of the three seeds is fluted pumpkin (72.0 - 86.0%) > cashewnut (74.3 - 82.9%) > Breadnut flour (71.3 - 78.3%). Processing techniques used reduced the antinutritional factors in the seeds and improve its IVPD when compared with the raw dried seed flours.

Key words: Processing antinutritional factors, *in vitro* protein digestibility, breadnut flour, fluted pumpkin

Introduction
The utilization of seed flours and plant proteins as functional ingredients in food system continue to be of research interest especially on soybean, peanuts, cottonseed and sunflower (Mcwatters, 1978; Rooney *et al.*, 1972; Lawhon and Cater, 1971; Lin *et al.*, 1974). Some of the seeds are high in antinutritional factors, which can limit their utilization in food system (Moran *et al.*, 1968; Wu and Inglett, 1974). Moran *et al.* (1968) showed that our traditional processing technique could effectively reduce the antinutritional factors in legumes and oil seeds. They also showed that Heat used in the commercial processing of seed products improves protein quality by destroying certain antinutritional factors.

Sprouting or germination has been reported to improve vitamins and protein quality of some cereals and legumes with reduction in antinutritional factors, (Kyler and McCready, 1975; Asiedu *et al.*, 1992). All over the world, fermented food provide an important part of human diet. Fermented foods and beverages provide about 20-40% of human food supply Campbell-Platt (1994). Traditional food fermentation is capable of improving the nutrients of the food, preserve it by generating acidic condition, detoxify and reduce cooking time of the food, Steinkraus (1995). Zamora and Veum (1979) reported an increase in weight, biological value

(BV), True Digestibility (TD) and Net Protein Utilization (NPU) values of rats fed on fermented soybean compared with the heat treated unfermented seeds. Breadnut, cashewnut and fluted pumpkin seeds are underutilized and may contain some antinutritional factors. There is limited information on processing effects on the antinutrients in the seeds especially our traditional food processing practices.

The aim of the study was to investigate processing effects on the antinutrittional factors and invitro protein digestibility of the seed flour in order to provide information that can enhance the utilization of the seeds.

Materials and Methods
Seed collection and processing: Mature fruits of breadnut were freshly harvested from a private garden at Okuta Elerinla, Akure. Cashewnuts were obtained from commodity support service (CSS), Ilesha Road, Akure, while fluted pumpkin fruits were harvested from private farms in the University campus. The seeds were extracted and processed as follows: The seeds were dehulled, adhering testa were removed, sliced, washed and dried. Part of the sliced seeds before drying were boiled for 1hour as described by Giami and Bekebain (1992) and dried. Parts of the boiled seeds were fermented naturally and oven dried Achinewhu, (1982). Fresh seeds were germinated as described by Giami

and Bakebain, (1992) and the seeds with sprout of about 1cm were dehulled, sliced, washed and dried Part of the raw dried seeds was roasted in an open cast iron at temperature of 75-85°C. The differently processed seeds were all dried at 50°C, milled, sieved to pass through 500µm sieves and packaged in polyethylene containers for further analysis.

Determination of trypsin inhibitor activity (TIA): The trypsin inhibitor activities were determined using the procedure of Kakade *et al.* (1969), as modified by Smith *et al.* (1980). Benzoyl-DL-arginine-P-nitro anilide hydrochloride (BAPNA) obtained from Zefa Labor Service Germany was used as substrate. Crystalline porcine pancreatic trypsin (type ZF 93615.0025) 40mg (Boehinge Bellane lowes) was obtained from Zefa labor service Germany and dissolved in 0.001M HCl such that standard trypsin solution contains 40µg trypsin.

Extraction of trypsin from flour samples: 1.0g of finely ground and sieved sample of each seed flour was defatted for 3 h using n-hexane. The sample was mixed with 50ml of 0.01M NaOH and the pH was adjusted to 9.5 using 0.1M NaOH or 0.1M HCl. The mixture was macerated in warring blender for 2 min and centrifuged for 10 min at 1,000 rpm. The extract from each sample was diluted with distilled water to obtain a dilution whereby 1ml extract produced trypsin inhibition activity of between 40- 60%. Such dilution was used.

Trypsin inhibitor activity determination: Each sample dilution was used with BAPNA substrate and trypsin solution as described by Kakade *et al.* (1969) at 37°C. The reaction was allowed to take place in water bath for 10 min and their absorbance read at 410nm against each sample blank.

Trypsin inhibitor activity (TIA) was calculated as:
TIA = [2.632 x D x A_1]/S = mg pure trypsin/g sample
D = Dilution factor
A_1 = change in absorbance (pure trypsin and sample extract)
S = sample mass (g)

Determination of phosphorus compounds: The phytic acid content of the seed flours was determined by extraction and precipitation as described by Young and Greaves, (1940) modified by Wheeler and Ferrel (1971). 8.0g of each flour sample was soaked in 200ml of 2% HCl for 3 h. The mixture was filtered and 50ml of the filtrate was titrated against standard $FeCl_3$ (0.00195 Fe^{3+} g/ml) using 0.3% ammonium thiocyanate indicator until a bluish yellow colour persisted. The iron in the precipitate was determined by the method of Makower (1970). A 4:6 Fe/P molecular ratio was used to calculate phytin phosphorus and phytic acid content.

Total phosphorus was determined spectrophotometrically (Pye Unicam) by the phosphor vanado molybdate method, AOAC (1990).

Determination of tannin content of the seed flours: The tannin content of the seed flours was determined by modifying the procedure of Makkar (1994). The seed flours were defatted using diethyl ether, ground and sieved through 500µm sieve. 0.2mg of the defatted flour was extracted with 10ml of 70% aqueous acetone Aletor, (1993) for 2 h in water bath at 30°C. The extract was centrifuged at 3,500 rpm for 20 min and 0.05ml of the supernatant was used.

Increasing concentration of standard tannic acid was prepared and 0.5ml folin-Ciocalteu reagent was added and their absorbance measured at 725nm against distilled water, using spectrophotometer. The absorbance of the various tannic acid concentrations was used to obtain a regression equation that was used to determine tannic acid in each sample extract. The regression equation obtained was
Y = 0.021x - 0.01;   r = 0.996
Y = absorbance; x = tannic acid (µg)
Tannic acid from each sample was determined and expressed as g/kg of the flour sample.

***In vitro* multienzyme protein digestibility (IVPD) determination:** The IVPD of the seed flours was determined using the procedure of Hsu *et al.* (1977).The enzymes used include porcine pancreatic trypsin (Z.F 93615.0025) bovine pancreatic chymotrypsin (ZF. 27270) and porcine intestinal peptidase (Z.F. 77163.0500) were purchased from Zefa laboservice GMBH Germany. The activity of the enzymes was initially determined before use by using them to digest casein. All the samples i.e. breadnut, cashewnut and fluted pumpkin seed flours were ground to fine powder and sieved. About 1.62g of each of the breadnut flours, 1.12g of each of cashewnut flour and 1.08g of fluted pumpkin seed flours were each dissolved in 50ml-distilled water to give sample suspension of 6.25mg protein/ml. Each sample suspension was adjusted to pH 8 and incubated in water bath at 37°C with constant stirring. Fresh multienzyme solution was prepared to contain 1.6 mg trypsin, 3.1mg chymotrypsin and 1.4mg peptidase dissolved in 1ml distilled water. The pH of enzyme solution was maintained at 8.0, 5ml of the multi enzyme solution was added to each sample suspension with constant stirring at 37°C. The pH of each sample suspension was recorded at 10 min and 15 min respectively after adding the enzyme solution. The IVPD was calculated using the equation proposed by Hsu *et al.* (1977).
Y = 210.464 - 18.103 x
Y = *In vitro* protein digestibility (%)
X = pH of sample suspension after 10 min and 15 min

Table 1: Processing Effects on the Antinutritional factors in full fat Bread nut seed Flours**

Antinutritional Factors	B1	B2	B3	B4	B5
Tannin (g/kg)	99.2±0.03$_a$	5.8±0.02$_c$	6.8±0.02$_b$	7.2±0.02$_b$	6.1±0.02$_c$
(% Change*)		(37.0)	(26.1)	(21.7)	(36.7)
Trypsin inhibitor Mg/g	8.1±0.22$_a$	6.5±0.18$_b$	0.9±0.03$_c$	8.1±0.22$_a$	6.0±0.16$_b$
(% Change)		(20.4)	(88.7)	(0.9)	(26.8)
Phytic acid (g/kg)	5.3±0.01$_a$	3.6±0.01$_c$	2.8±0.01$_d$	3.6±0.01$_c$	4.6±0.01$_b$
(% Change)		(32.1)	(47.2)	(32.1)	(13.2)

*% change due to processing. ** Values with different subscript on the same row are significant (P< 0.05). B$_1$ = Raw dried breadnut; B$_2$ = Boiled breadnut B$_3$ = Fermented breadnut. B$_4$ = Germinated breadnut B$_5$ = Roasted breadnut

Table 2: Processing Effects on the Antinutritional Factors in full fat Cashewnut Flours

Antinutritional Factors	C1	C2	C3	C4	C5
Tannin (g/kg)	13.3±0.04$_a$	5.1±0.01$_e$	6.7±0.02$_d$	11.5±0.03$_b$	8.5±0.02$_c$
(% Change*)		(61.7)	(49.6)	(13.5)	(36.1)
Trypsin Inhibitor (mg/g)	2.5±0.07$_a$	1.1±0.03$_c$	0.8±0.02$_d$	1.7±0.05$_b$	1.0±0.03$_c$
(% Change*)		(57.1)	(67.1)	(34.5)	(58.7)
Phytic acid (g/kg)	9.9±0.03$_a$	6.3±0.02$_c$	6.0±0.02$_c$	7.1±0.02$_b$	6.4±0.02$_c$
(% Change*)		(36.4)	(39.4)	(28.4)	(35.4)

* % change due to processing. ** values with different subscript on the same row are significant (P< 0.05). C$_1$ = Raw dried Cashewnut; C$_2$ = Boiled Cashewnut C$_3$ = Fermented Cashewnut. C$_4$ = Germinated Cashewnut C$_5$ = Roasted Cashewnut

Table 3: Processing Effects on the antinutritional factors in full-fat Fluted Pumpkin seed Flours **

Antinutritional Factors	F1	F2	F3	F4	F5
Tannin (g/kg)	19.1±0.05$_a$	7.5±0.02$_d$	9.8±0.03$_c$	14.0±0.04$_b$	9.9 ±0.03$_c$
(% Change*)		(60.7)	(48.7)	(26.7)	(48.2)
Trypsin Inhibitor (mg/g)	11.0±0.03$_a$	Ndc	Ndc	0.7±0.02$_b$	NDc
(% Change)		(100)	(100)	(94.0)	(100)
Phytic acid (g/kg)	13.8±0.04$_a$	4.3±0.01$_c$	2.8±0.01$_d$	6.4±0.02$_b$	6.0±0.02$_b$
(% Change)		(64.8)	(79.7)	(53.6)	(56.5)

* % change due to processing. ND=Not detected (0.05µg). ** values with different subscript on the same row are significant (P< 0.05)
F$_1$ = Raw dried Fluted Pumpkin ; F$_2$= Boiled Fluted Pumpkin seed F$_3$= Fermented Fluted Pumpkin seed. F$_4$= Germinated Fluted Pumpkin seed F$_5$ = Roasted Fluted Pumpkin seed

All the other reagents used (BDH) chemicals Ltd Poole England were of analytical grade

Statistical analysis: Determinations were made in triplicates; errors were calculated as standard errors of the mean (SEM) and analysis of variance (ANOVA) in SPSS 10 computer programme was used to analyze the results. Means were separated using Duncan multiple range. Significance was accepted at the 0.05 level of probability.

Results and Discussion
Table 1, 2 and 3 show the processing effects on some antinutritional factors in breadnut, cashewnut and fluted pumpkin seed flours respectively.

Phytic acid: The phytic acid content of breadnut, cashewnut and fluted pumpkin seed flours ranged between 2.8-5.3g/kg, 6.0-9.9g/kg and 2.8-13.8g/kg respectively. Comparing the phytic acid contents of cashewnut, and fluted pumpkin seed flours with the phytic acid content of some oil seeds, it is within the

range of values reported for peanut (1.36%),Fardiaz and Markakis (1981) and the value reported for dehulled and whole soybeans (1.07-1.65)%, Sutardi and Buckle (1985); Paredes-Lopez and Harry (1989) but it is higher than the (0.18%) value reported for raw locust beans , (Eka, 1980).
Considering the processing effects on the phytic acid content of the seed flours, boiling, fermentation, germination and roasting reduced the phytic acid contents of breadnut, cashewnut and fluted pumpkin seed flours by 32.1, 47.2, 32.1 and 13.2%; 36.6, 39.4, 28.3 and 35.4%; 64.8, 79.7, 53.6 and 56.5% respectively. From the above results, processing significantly (P≤ 0.05) reduced the phytic acid content of the three seeds. Fermentation is the most effective processing technique that reduced phytic acid in the seed flours. The result agreed with the report of Amoa and Muller, (1976) who reported 31.1% reduction in phytic acid content of kenkey (fermented maize) and 45.5% reduction reported by Sudarmadji and Markakis, (1977) in fermentation of common beans to tempe. It is also consistent with the result of Fardiaz and MarKakis, (1981) and Sutardi and

Table 4: Processing effects on the total phosphorus, phytin phosphorus and percentage of total that is phytin and digestible phosphorus in breadnut flours

Samples	Total phosphorus (g/kg)	Phytin P (g/kg)	Phytic acid (g/kg)	Phytin P as % of total phosphorus	Digestible P as % of total phosphorus
B1	$6.7\pm0.01_b$	1.5 ± 0.03	$5.3\pm0.02_a$	$22.4\pm0.3_a$	$77.6\pm0.3_e$
B2	$5.2\pm0.02_c$	1.0 ± 0.01	$3.6\pm0.02_c$	$19.2\pm0.2_c$	$80.8\pm0.5_c$
% Change*	(22.4)	(33.3)	(32.1)	(14.1)	(4.1)
B3	$7.8\pm0.03_a$	0.8 ± 0.01	$2.8\pm0.02_d$	$10.3\pm0.2_e$	$89.7\pm0.1_a$
% Change	(16.4)	(46.7)	(47.2)	(54.2)	(15.6)
B4	$6.0\pm0.06_{bc}$	1.0 ± 0.06	$3.6\pm0.02_c$	$16.7\pm0.6_d$	$83.3\pm0.2_b$
% Change	(10.5)	(33.3)	(32.1)	(25.5)	(7.4)
B5	$6.4\pm0.02_b$	1.3 ± 0.01	$4.6\pm0.01_b$	$20.3\pm0.3_b$	$79.7\pm0.6_d$
% Change	(4.5)	(13.3)	(13.2)	(9.3)	(2.7)

* % change due to processing. ** values with different subscript on the same row are significant (P< 0.05). B_1 = Raw dried breadnut; B_2 = Boiled breadnut B_3 = Fermented breadnut. B_4 = Germinated breadnut B_5 = Roasted breadnut

Table 5: Processing effects on the total phosphorus, phytin phosphorus and percentage of total that is phytin and digestible phosphorus in cashewnut flours

Samples	Total phosphorus (g/kg)	Phytin P (g/kg)	Phytic acid (g/kg)	Phytin P as % of total phosphorus	Digestible P as % of total phosphorus
C1	$4.9\pm0.02_b$	2.8 ± 0.01	$9.9\pm0.03_a$	$57.1\pm0.3_a$	$42.9\pm0.2_d$
C2	$4.1\pm0.01_d$	1.8 ± 0.05	$6.3\pm0.03_b$	$43.2\pm0.2_b$	$56.8\pm0.6_c$
% Change*	(16.3)	(36.8)	(36.4)	(33.3)	(32.6)
C3	$5.2\pm0.01_a$	1.7 ± 0.05	$6.0\pm0.12_b$	$32.7\pm0.3_d$	$67.3\pm0.5_a$
% Change	(6.1)	(39.3)	(39.4)	(42.8)	(57.0)
C4	$4.6\pm0.06_c$	$2.0\pm0.0.01$	$7.1\pm0.04_b$	$43.5\pm0.2_b$	$56.5\pm0.3_c$
% Change	(6.1)	(28.7)	(28.3)	(23.9)	(31.9)
C5	$4.5\pm0.05_c$	1.8 ± 0.03	$6.4\pm0.01_b$	$40.0\pm0.2_c$	$60.0\pm0.5_b$
% Change	(8.2)	(35.7)	(35.4)	(30.0)	(40.0)

* % change due to processing. ** values with different subscript on the same row are significant (P< 0.05). C_1 = Raw dried Cashewnut; C_2 = Boiled Cashewnut C_3 = Fermented Cashewnut. C_4 = Germinated Cashewnut C_5 = Roasted Cashewnut

Buckle, (1985) who reported 48-96.3% and 54.77% reduction in phytic acid content of peanut and soybeans respectively. Processing especially fermentation has been reported to reduce phytic acid content of cereals, legumes and tubers as a result of the activity of the endogenous phytases from both raw ingredient and inherent micro organisms which hydrolyse phytic acid in many fermented food preparation into inositol and orthophosphate (Reddy and pierson, 1994; Sandberg and Andlid, 2002). When the three seeds are compared for phytic acid content, fluted pumpkin (2.8-13.8g/kg)> cashewnut (6.0-9.9g/kg)> Breadnut (2.8-5.3g/kg).

Tannin: The tannin content of breadnut, cashewnut and fluted pumpkin flours ranged between 0.6-0.9g/kg; .5.1-10.3g/kg and 7.5-19.1g/kg respectively. The tannin content of the seed flours is within the range of the values reported for some undehulled common beans 0.95%, Paredes-Lopez and Harry, (1989). Boiling, fermentation, germination and roasting reduced the tannin content of breadnut, cashewnut and fluted pumpkin seed flours by 37.0, 26.1, 21.7 and 36.7%;

61.7, 49.6, 13.5 and 36.1%; 60.7, 48.7, 26.7 and 48.2% respectively. Boiling was the most effective processing technique to reduce the tannin content of the seed flours. Reddy and Pierson (1994), reported that dehulling and cooking eliminated more than 90% of the tannin content in soybean due to their predominance in seed coats, while dehulling, cooking and fermentation were generally reported to reduce tannin content of cereals and other foods, Salunkhe *et al.* (1990). Reduction in tannin due to processing might have been caused by the activity of polyphenol oxidase or fermented micro flora on tannins (Reddy and Pierson, 1994).

Comparing the three seeds, the tannin content of fluted pumpkin, (7.5-19.1g/kg) > cashewnut (5.1-13.3g/kg)> breadnut (5.8-9.2g/kg).

Trypsin ihibitor ativity (TIA): The TIA of breadnut, cashewnut and fluted pumpkin (Table 1, 2 and 3) ranged between 0.9-8.1mg/g; 0.08-2.5mg/g and 0-11mg/g respectively. Boiling, fermentation, germination and roasting, reduced the trypsin inhibitor activity of the seed flours by 20.4, 88.7, 0.9 and 26.8%; 57.1, 67.1, 34.5, and

Table 6: Processing effects on the total phosphorus, phytin phosphorus and percentage of total that is phytin and digestible phosphorus in fluted pumpkin seed flours

Samples	Total phosphorus (g/kg)	Phytin P (g/kg)	Phytic acid (g/kg)	Phytin P as % of total phosphorus	Digestible P as % of total phosphorus
F1	$10.0\pm0.2_b$	$3.9\pm0.02_a$	$13.8\pm0.2_a$	$39.0\pm0.2_a$	$61.0\pm0.6_d$
F2	$5.6\pm0.02_d$	$1.2\pm0.01_c$	$4.3\pm0.01_c$	$21.4\pm0.2_c$	$78.6\pm0.6_b$
% Change*	(44.0)	(69.2)	(68.8)	(45.1)	(28.8)
F3	$11.5\pm0.08_a$	$0.8\pm0.01_{cd}$	$2.8\pm0.02_d$	$7.0\pm0.02_e$	$93.0\pm0.3_a$
% Change	(15.0)	(79.5)	(79.7)	(82.1)	(52.5)
F4	$6.3\pm0.3_d$	$1.8\pm0.01_b$	$6.4\pm0.02_b$	$28.6\pm0.4_b$	$71.4\pm0.4_c$
% Change	(37.0)	(53.8)	(53.6)	(26.7)	(17.7)
F5	$8.5\pm0.28_c$	$1.7\pm0.01_b$	$6.0\pm0.02_b$	$20.0\pm0.6_d$	$80.0\pm0.1_b$
% Change	(15.0)	(56.4)	(56.5)	(48.7)	(31.1)

* % Change due to processing. ** Values with different subscript on the same row are significant (P< 0.05). F_1 = Raw dried Fluted Pumpkin; F_2 = Boiled Fluted Pumpkin seed F_3= Fermented Fluted Pumpkin seed. F = Germinated Fluted Pumpkin seed F = $_5$Roasted Fluted Pumpkin seed

58.7%; 100, 100, 94 and 100% respectively. Fermentation was the most effective processing technique to reduce the TIA in the seed flours. The results obtained in this work agreed with the observations of the previous workers (Paredes- Lopez and Harry, 1989; Roozen and De Groot, 1985) on cooked and fermented soybeans and common beans where reduction in TIA of between 91.4 - 99.9% and 52% were reported for fermented soybean and hot soaked cowpea respectively. Comparing the three seeds for TIA, Fluted Pumpkin (0.0-11.0mg/g) > Breadnut (0.9-8.1mg/g) > Cashewnut (0.8-2.5mg/g) flour.

Phosphorus compounds: Table 4, 5 and 6 show that phytin phosphorus, as a percentage of the total phosphorus in the raw dried seed flours was very high when compared with the processed samples. This may make the phosphorus in the raw dried seeds indigestible to humans due to their inability to synthesize phytase enzyme, Balogun, (1989). Processing affect the phosphorus compounds in the seed flours. Boiling, germination and roasting reduced the total phosphorus content of breadnut, cashewnut and fluted pumpkin by 22.4, 10.5 and 4.5%; 16.3, 6.1 and 8.2%; 44, 37 and 15%. Fermentation increased it by 16.4, 6.1 and 15.0% respectively.

The reduction in total phosphorus due to boiling was attributed to leaching, Ologhobo and Fetuga (1984), while the increase due to fermentation may be due to the activity of the phytase enzyme that hydrolyses phosphorus compound during fermentation. Processing significantly (P ≤ 0.05) reduced the phytin phosphorus in the seed flours. Boiling fermentation, germination and roasting reduced the phytin phosphorus of breadnut, cashewnut and fluted pumpkin seed flours by 33.3, 46.7, 33.3 and 13.3%; 36.8, 39.3, 28.3 and 35.4%; 69.2, 79.5, 53.8 and 56.4% respectively. Fermentation is the most effective processing technique for reducing phytin

phosphorus. During germination, phytase and phosphatase activities increased, Ologhobo and Fetuga, (1984), thereby decreasing the phytin phosphorus while most of the phytin phosphorus was reported to be located at the sprouts Asiedu *et al.* (1993), thereby reducing the amount present in the seeds. Processing significantly increased the digestible or available phosphorus in the seed flours. The raw dried seeds have the least digestible phosphorus while the fermented seeds had the highest. Thus, boiling, germination, fermentation and roasting may be used to reduce phytin phosphorus in the seeds but the most effective method is fermentation.

In vitro **multi-enzyme protein digestibility (IVPD):** From Table 7, 8 and 9 the IVPD of breadnut, cashewnut and fluted pumpkin flours ranged between 71.3 - 78.3%, 74.3 - 82.4% and 72.0-86.5% respectively. Processing affected the IVPD of the three seed flours. Boiled samples have the highest digestibility values while the raw or the germinated samples have the least.

The IVPD of the oil seeds, cashewnut and fluted pumpkin compared favourably with the digestibility of some oil seeds like winged bean protein (84.7%), soy isolate (87.7 - 89.6%) and cotton meal (85.3%) Hsu *et al.* (1977). Heat processing, especially, moist heat have been reported to improve the digestibility of proteins by destroying protease inhibitors and opening up of the protein structure through denaturation, (Tannenbaum 1974; Abbey and Berezi, 1988). This might explain why the boiled samples, B_2, C_2 and F_2 showed highest digestibility. Natural fermentation has also been reported to cause significant improvement in IVPD of pearl millet, Elyas *et al.* (2002), due to the activity of proteolytic enzymes and reduction in anti nutritional factors. It was also observed that the roasted samples, B_5, C_5 and F_5 had lower digestibility than raw samples (B_1, C_1 and F_1) despite the heat processing applied. The

Table 7: Effect of Processing on the In-vitro multi enzyme protein digestibility of Breadnut flours

Sample	pH 10 min	% Digestibility	pH 15 min	% (15 min) Digestibility
B1	7.69	$71.3\pm1.0_e$	7.70	$71.1\pm3.0_e$
B2	7.30	$78.3\pm1.0_a$	7.32	$78.0\pm2.5_a$
B3	7.38	$76.9\pm2.5_b$	7.52	$74.3\pm1.5_b$
B4	7.51	$74.5\pm1.5_c$	7.53	$74.2\pm1.5_c$
B5	7.55	$73.8\pm2.5_d$	7.68	$71.4\pm1.5_d$

Values with different subscripts on the same column are significant P = 0.05. B_1 = Raw dried breadnut; B_2 = Boiled breadnut B_3 = Fermented breadnut. B_4 = Germinated breadnut B_5 = Roasted breadnut

Table 8: Effect of Processing on the In-Vitro multi enzyme Protein digestibility of cashewnut flour

Sample	pH 10 min	% Digestibility	pH 15 min	% (15 min) Digestibility
C1	7.25	$79.2\pm1.5_c$	7.45	$75.6\pm2.0_c$
C2	7.05	$82.8\pm2.0_a$	7.09	$82.1\pm3.0_a$
C3	7.12	$81.6\pm1.0_b$	7.10	$81.9\pm1.5_a$
C4	7.52	$74.3\pm1.5_e$	7.56	$73.6\pm1.0_d$
C5	7.29	$78.5\pm1.5_d$	7.31	$78.1\pm2.0_b$

Values with different subscripts on the same column are significant P = 0.05. C_1 = Raw dried Cashewnut; C_2 = Boiled Cashewnut C_3 = Fermented Cashewnut. C_4 = Germinated Cashewnut C_5 = Roasted Cashewnut

Table 9: Effect of Processing on the In-vitro multi enzyme protein digestibility of fluted pumpkin seed flours

Sample	pH 10 min	% Digestibility	pH 15 min	% (15 min) Digestibility
F1	7.30	$78.3\pm1.0_c$	7.28	$78.7\pm1.5_c$
F2	6.85	$86.5\pm2.0_a$	6.95	$84.7\pm2.5_b$
F3	6.88	$85.9\pm2.0_b$	6.93	$85.0\pm1.5_a$
F4	7.65	$72.0\pm1.0_d$	7.70	$71.1\pm1.5_e$
F5	7.31	$78.1\pm3.5_c$	7.32	$78.0\pm2.5_d$

Values with different subscripts on the same column are significant P = 0.05. F_1 = Raw dried Fluted Pumpkin ; F_2 = Boiled Fluted Pumpkin seed F_3 = Fermented Fluted Pumpkin seed. F_4 = Germinated Fluted Pumpkin seed F_5 = Roasted Fluted Pumpkin seed

result agreed with the observations of some previous workers (Hsu *et al.*, 1977 and Tannenbaum, 1974) who reported that dry heat processing reduced protein digestibility due to non-enzymic browning. Maillard reaction from starch hydrolysis and the proteins as well as the thermal cross linking that occurred during roasting reduced IVPD. The order of the IVPD of the three seeds as affected by processing is breadnut: $B_2 > B_3 > B_4 > B_5 > B_1$; Cashewnut; $C_2 > C_1 > C_5 > C_4$ and fluted pumpkin seed, $F_2 > F_3 > F_1 > F_5 > F_4$

When the IVPD of fluted pumpkin obtained in this work (72.0-86.5%) is compared with the in vivo result of rat feeding reported earlier (84.1-88.2%) Achinewhu and Isichei, (1990) and 78.31-84.4% reported by Longe *et al.* (1983), there was a perfect degree of correlation of 1.00 between the invitro and in vivo protein digestibility and they followed the same trend.

Generally, the oil seeds (Cashewnut and fluted pumpkin) showed the same trend of digestibility. The order of invitro protein digestibility of the three seeds when compared is; fluted pumpkin (72.0-86.5%) > cashewnut (74.3-82.8%) > breadnut (71.3-78.3%).

Conclusion: Breadnut, cashewnut and fluted pumpkin seed flours contain some level of phytic acid, trypsin inhibitor, and tannins. Processing especially boiling and fermentation can effectively reduce the antinutritional factors with significant increase in the *in vitro* multienzyme protein digestibility.

References

Abbey, B.W. and P.E. Berezi, 1988. Influence of processing on the digestibility of African yam bean (*Sphenostylis stenocarpa*) flour. Nutrition reports International 32: 819-827.

Achinewhu, S.C., 1982. Composition and food potential of African oil bean (*Pentaclethra macrophylla*) and Velvet Bean (*Muena urines*). J. Food Sci., 47: 1736-1737.

Achinewhu, S.C. and M.O. Isichei, 1990. The nutritional evaluation of fermented fluted pumpkin seeds (*Telfairia occidentalis* Hook). Discovery and Innovation, 2: 62-65.

Aletor, V.A., 1993. Allelochemicals in plant food and feeding stuffs (1) Nutritional, biochemical and physiopathological aspects in animal production. J Vet. Human Toxicol., 35: 57-67.

Amoa, B. and H.G. Muller, 1976. Studies on Kenkey with particular reference to calcium and phytic acid. Cereal Chem., 53: 365-375.

Asiedu, M., R. Nilsen, O. Lie and E. Lied, 1992. Effect of processing (sprouting and/or fermentation) on sorghum and maize I proximate composition, minerals and fatty acids. Food Chem., 47 submitted.

Asiedu, M., E. Lied, R. Nilsen and K. Sandnes, 1993. Effect of Processing (sprouting and fermentation) on sorghum and maize II. Vitamins and amino acid composition. Biological utilization of maize protein. Food Chem., 48: 20 204.

Association of Official Analytical Chemist (A.O.A.C.), 1990. Official Methods of Analysis 15th edition Association of Official Analytical Chemists, Washington DC.

Balogun, A.M., 1989. Anti-nutritional components in some lesser-known leguminous crop seeds in Nigeria. Biological Wastes, 28: 303-308.

Campbell-Platt, G., 1994. Fermented Foods - a world perspective, Food Res. Int., 27: 253-257.

Eka, O.U., 1980. Effect of fermentation on the nutrient status of locust beans. Food Chem., 5: 303-308.

Elyas, S.H.A., A.H. Tinay, N.E. Yousif and E.A.E. Elsheikh, 2002. Effect of natural fermentation on nutritive value and *in vitro* protein digestibility of Pearl millet. Food Chem., 78: 75-79.

Fardiaz, D. and P. Markakis, 1981. Degradation of phytic acid in oncom (Fermented Peanut Press Cake) J. Food Sci., 46: 523-525.

Giami, S.Y. and D.A. Bekebain, 1992. Proximate Composition of Processed full-fat fluted pumpkin (*Telfairia occidentalis*) seed flour. J. Sci. Food Agri., 59: 321-325.

Hsu, H.W., D.L. Vavak, L.D. Satterlee and G.A. Miller, 1977. A Multi- enzyme Technique for estimating protein digestibility J. Food Sci., 42: 1269-1273.

Kakade, M.L., N. Simons and L.E. Liener, 1969. An evaluation of natural Vs synthetic substrates for measuring the anti tryptic activity of soybean samples. Cereal Chem., 46: 518-526.

Kyler, A.M. and R.M. McCready, 1975. Nutrient in seeds and sprouts of Alfalfa lentils, mung beans and soybeans. J. Food Sci., 40: 1008-1013.

Lawhon, J.T. and C.M. Cater, 1971. Effect of processing method and pH of precipitation on the yields and functional properties of protein isolates from glandless cottonseed. J. Food Sci., 39: 372-376.

Lin, M.J.Y., E.S. Humbert and F.W. Sosulski, 1974. Certain functional properties of sunflower meal products Food Sci., 42: 368-370.

Longe, O.G., G.O. Farinu and B.L. Fetuga, 1983. Nutritional value of the fluted pumpkin (*Telfairia occidentalis*) J. Agri. Food Chem., 31: 989-992.

Makower, R.V., 1970. Extraction and determination of phytic acid in beans (*Phaseolus vulgaris*) Cereal Chem., 47: 288 - 292.

Makkar, H.P.S., 1994. Quantification of tannins. A laboratory manual. International Centre for Agricultural Research in the Dry Area (ARDA) Aleppo Syria.

Mcwatters, K.H., 1978. Cookie baking properties of defatted peanut, soybean and field pea flours. Cereal Chem., 55: 853-857.

Moran, Jr. E.T., J.D. Summers and E.J. Bass, 1968. Heat processing of wheat germ meal and its effect on utilization and protein quality for the growing chick; roasting and autoclaving. Cereal Chem., 45: 304-308.

Ologhobo, A.D. and B.L. Fetuga, 1984. Distribution of phosphorus and phytate in some Nigerian varieties of legumes and some effects of processing. J. Food Sci., 49: 199-201.

Paredes-Lopez, O. and G.I. Harry, 1989. Changes in selected chemical and antinutritional components during tempeh preparation using fresh and hardened common beans. J Food Sci., 54: 968-970.

Reddy, N.R. and M.D. Pierson, 1994. Reduction in antinutritional and toxic components in plant foods by fermentation. Food Res. Int., 27: 281-290.

Rooney, L.W., C.B. Gustafson, S.P. Clark and C.M. Cater, 1972. Comparison of the baking properties of several oil seed flours J Food Sci., 37: 14-21.

Roozen, J.P. and J. De Groot, 1985. Electrophoresis and assay of trypsin inhibitors in different stages of tempeh production. J. Food Biochem., 9: 37-48.

Sandberg, A.S. and T. Andlid, 2002. Phytogenic and microbial phytases in human nutrition. Int. J. Food Sci., Tec., 37: 823-833.

Salunkhe, D.K., J.K. Chavan and S.S. Kadam, 1990. Dietary Tannins Consequences and Remedies CRC Press Boca. Raton FL.

Smith, C., W.V. Megen, L. Twaalfhaven and C. Hitchock, 1980. The determination of Trypsin Inhibitor Levels in Food Stuffs. J. Sci. Food Agri., 34: 341-350.

Sudarmadji, S. and P. Markakis, 1977. The phytate and phytase of soybean tempeh. J. Sci. Food Agri., 28: 381-383.

Sutardi, A. and K.A. Buckle, 1985. Phytic acid changes in soybeans fermented by traditional inoculum and six strains of *Rhizopus oligosporus*. J. Appl. Bacteriol., 58: 539-543.

Steinkraus, K.H. Ed, 1995. Handbook of Indigenous fermented Foods New York USA. Marcel Dekker Inc, pp: 776.

Tannenbaum, S., 1974. Industrial Processing. In "Nutrition in Processed Food Proteins". Publishing Sciences Group Inc Acton MA.

Wheeler, E.L. and R.E. Ferrel, 1971. A method of phytic acid determination in wheat. Cereal Chem., 48: 312-316.

Wu, Y.U. and G.E. Inglett, 1974. Denaturation of plant proteins related to functionality and food applications. A review. J. Food Sci., 38: 218-225.

Young, S.M. and J.E. Greaves, 1940. Influence of variety and treatment on phytin content of wheat. Food Res., 5: 103-108.

Zamora, R.G. and T.L. Veum, 1979. The nutritive value of dehulled soybean fermented with *Aspergillus, Oligosporus* or *Rhizopus* as evaluated by rats J. Nutr., 109: 1333-1339.