Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 88 (2004) 397–404
Food Chemistry
www.elsevier.com/locate/foodchem

Effect of fermentation on the seed proteins, nitrogenous constituents, antinutrients and nutritional quality of fluted pumpkin (*Telfairia occidentalis* Hook)

S.Y. Giami

Department of Food Science and Technology, Rivers State University of Science and Technology, Port Harcourt, Nigeria

Received 17 June 2003; received in revised form 21 January 2004; accepted 21 January 2004

Abstract

The effect of fermentation, for 7 days, on levels of nitrogenous constituents, protein fractions, antinutrients and protein quality of fluted pumpkin (*Telfairia occidentalis* Hook) seed was investigated. Protein quality was evaluated using weanling albino rats fed diets which were formulated to supply 10% protein using fermented and unfermented fluted pumpkin seed samples, with casein as a control. The non-protein nitrogen gradually increased and the protein nitrogen decreased during fermentation. Albumin and globulin fractions were found to be the major seed proteins of fluted pumpkin seeds, constituting about 59% of the total protein of the unfermented seeds. These protein fractions (albumin and globulin) increased during fermentation, reaching their maximum levels (34.9% and 30.6% of total extractable protein, respectively), on the 5th day, but declined thereafter. Fermentation significantly ($p < 0.05$) increased crude protein and in vitro protein digestibility but decreased polyphenol and phytic acid contents of the seeds. The values obtained for protein efficiency ratios, net protein ratios and true digestibilities of diets formulated with pumpkin seeds fermented for 5 days were similar to those of casein.

Keywords: Antinutrients; Fermentation fluted pumpkin; Protein quality; Seed proteins

1. Introduction

Fluted pumpkin (*Telfairia occidentalis* Hook) seed has about 27% protein and 54% fat and is potentially valuable as a high protein oil seed for human and animal food in Nigeria (Longe, Farinu, & Fetuga, 1983). Besides being boiled and eaten as a vegetable, the seeds are fermented and used as a flavouring agent or protein supplement in a variety of local foods (Achinewhu, 1987; Banigo & Akpapunam, 1987). However, the usefulness of fluted pumpkin seed as a protein source for human food is limited by the presence of antinutrients which have been shown to have detrimental physiological effects on growing rats and chicks, thereby limiting its nutritional value (Achinewhu & Isichei, 1990; Nwokolo & Sim, 1987). Although many aspects of the functional (Giami & Bekebain, 1992; Giami & Isichei, 1999), nutritional (Achinewhu & Isichei, 1990), microbiological (Barber, Ibiama, & Achinewhu, 1989) and biochemical (Achinewhu, 1986) changes taking place within the seed during fermentation have been examined, information on the effect of fermentation on changes in the levels of the seed proteins, in vitro protein digestibilities, types and quantities of antinutrients in the seed is lacking.

During fermentation, seeds are reported to undergo pronounced compositional changes as a result of proteolytic processes within the seeds, leading to degradation and alteration of storage proteins (Biehl, Wewetzer, & Passern, 1982). Compositional alterations, that occur during fermentation, include changes in protein fractions, such as albumin, globulin, prolamine and glutelin (El-Khalifa & El-Tinay, 1994). It has been reported that each protein fraction tends to have a characteristic amino acid composition and the relative proportion of

E-mail address: sonygiami@hotmail.com (S.Y. Giami).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.01.064

each fraction in the seed strongly affects the nutritional quality of the total seed protein (Johnson & Lay, 1974). A positive correlation between the albumin content and nutritive value of peas and between glutelin content and biological value, lysine and tryptophan levels of maize has also been reported (Bajaj et al., 1971; Mertz, Veron, & bates, 1965). This has led to the idea of using measures of protein fractions as indicators of protein quality. Hence, knowledge of the changes which occur in protein fractions during fermentation may be useful in assessing nutritive value of the seed.

In a previous study (Giami & Bekebain, 1992), fermentation was observed to increase the crude protein content of fluted pumpkin seed, although it was not determined whether the increase was a result of the synthesis of protein nitrogen (PN) or non-protein nitrogen (NPN). Fermentation has been reported to improve the nutritive value of food legumes and cereals by decreasing the levels of antinutrients (Dhankher & Chauhan, 1987; Eka, 1980) and increasing protein digestibility (Chavan, Chavan, & Kadam, 1988; Taylor & Taylor, 2002). The aim of this study was to examine the effect of fermentation on changes in protein fractions, nitrogenous constituents, antinutrients (polyphenol and phytic acid) and protein quality of fluted pumpkin seed.

2. Materials and methods

2.1. Materials

Fluted pumpkin (*Telfairia occidentalis* Hook) fruits were obtained from local markets in Port Harcourt. The seeds were separated from the pulp and husks and used in the experiments. Triplicate samples were selected and subjected to fermentation. Pancreatin and porcine pepsin powder, manufactured by Sigma Chemical Company, St. Louis M.O. USA, were obtained from local scientific stores in Port Harcourt. Weanling male albino rats (Wistar strain) were obtained from the animal colony of the University of Port Harcourt, while vitamin and mineral premixes were supplied by Rhodia Nig. Ltd. Lagos, Nigeria. All other reagents used (BDH Chemicals Ltd., Poole England) were of analytical grade.

2.2. Fermentation

The traditional natural fermentation method described by Barber et al. (1989) was used. Seeds, with intact seed coats, were boiled in tap water (1:10 w/v) in a covered stainless steel pot for 1 h to soften the seed coat and aid dehulling. The seed coats were removed using hand pressure and the cotyledons were ground to a paste with NaCl (1 g/kg); then the paste was wrapped (40–50 g per pack) in flame-blanched plantain leaves, to provide a warm humid atmosphere, and left at room temperature (28 ± 1 °C) for 7 days to ferment.

2.3. Sample preparation

Fermented samples were collected at one day intervals until the end of fermentation. Fermented paste and unfermented seeds (raw, dehulled) were divided into two groups. One group; was oven-dried (60 °C, 24 h) in a hot air fan oven (Model QUB 305010G, Gallenkamp, UK), ground using a laboratory mill (Numex Pep Grinding Mill, India) and screened through a 0.25 mm British standard sieve (Model BS 410, Endecotts Ltd., London, UK). These flour samples were used for estimation of nitrogenous constituents, antinutrients and proximate composition of the seeds. Flours to be used in test diet formulations for rat studies were defatted by solvent extraction in a Soxhlet apparatus (Tecator Inc., Co, USA) for 8 h using *n*-hexane. Acetone powder was prepared from the second group using the method described by Shastry and John (1991). Samples (200 ± 1 g) were homogenized (Kenwood A 907 D, 5000 rpm) with 100 ml cold (−8 °C) 80% (v/v) aqueous acetone for 1 min, filtered through Whatman No. 1 filter paper and the residue was air-dried at room temperature (2 h) and stored at −20 °C. This acetone powder was used for protein fractionation, estimation of protein fractions and in vitro protein digestibility.

2.4. Protein fractionation

Solubility fractionation of the seed protein was carried out according to the method of Shastry and John (1991). This method was modified by using ethanol instead of isopropanol to extract prolamine, in accordance with the procedure outlined by El-Khalifa and El-Tinay (1994). Five grammes of acetone powder were suspended in 100 ml of extracting solvent and the proteins extracted successively at room temperature for 30 min with continuous shaking on a mechanical shaker (Model SGL 700–010V, Griffin and George Ltd. UK) with the following solvents:

(a) distilled water to extract albumin,
(b) 1.0 M NaCl to extract globulin,
(c) 70% (v/v) ethanol to extract prolamine,
(d) 0.1 M NaOH to extract glutelin.

Repeated extractions with the different solvents (twice each) were done. After each extraction, the slurry was centrifuged at 5000 rpm for 30 min. The supernatant was collected and the residue was redispersed in 100 ml of extracting solvent and used for successive extraction. For each extracting solvent, the supernatants were combined to give total extract. The protein content of each extract was determined by the Biuret method (Frais, 1972), using a reference to a standard graph based on casein.

2.5. Chemical analyses

Proximate compositions of fermented and unfermented samples were determined according to AOAC (1984) procedures. The factor 5.30 was used for conversion of nitrogen to crude protein. Carbohydrate content was calculated by difference. For the estimation of nitrogenous constituents, the total nitrogen (TN) content of the sample was determined by Kjeldhal digestion (AOAC, 1984). Non-protein nitrogen was determined as the nitrogen in the filtrate recovered after having precipitated the protein from solution with a 3% (w/v) copper acetate solution, as described by Osborne and Voogt (1978). The protein nitrogen was calculated by difference (TN-NPN).

Polyphenols were determined using the vanillin–H_2SO_4 assay described by Wilson and Blunden (1983) with reference to a standard graph based on phloroglucinol. The results are expressed as mg phloroglucinol equivalents per 100 g dry flour weight.

For the determination of phytic acid, a combination of two methods was used. The extraction and precipitation of phytic acid were performed according to the method of Wheeler and Ferrel (1971); iron in the precipitate was measured by the AOAC (1984) method. A 4:6 Fe/P molecular ratio was used to calculate phytic acid content. The procedure outlined by Saunders, Connor, Booth, Bickoff, and Kohler (1973) was used to assess in vitro protein digestibility with pepsin and pancreatin. The nitrogen contents of the sample and of the indigestible residue were determined by the AOAC (1984) method, and protein digestibility was calculated as digestible protein, expressed as a percentage of the total protein; pH was measured with a pH meter (PYE Unicam, Model 290).

2.6. Biological evaluation of protein quality

2.6.1. Formulation of diets

Protein quality was evaluated by a rat bioassay. Ten percent protein diets were prepared, based on the formulation of the AOAC (1984), as described in a previous paper (Giami, 2002). The diets were formulated using defatted flour samples of fluted pumpkin seeds which were subjected to fermentation for 0 day (sample A), 1 day (sample B), 2 days (Sample C), 3 days (Sample D), 4 days (Sample E), 5 days (Sample F), 6 days (Sample G), and 7 days (Sample H). In addition to the eight test diets, a casein (control) diet (sample I) and a protein-free (basal) diet (Sample J) were also prepared. The diets had the composition shown in Table 1.

2.6.2. Protein efficiency ratio

Weanling male albino rats of the Wistar strain, 28 days old, and weighing between 34 and 37 g were grouped by randomized block design into ten groups of ten replicate rats on the basis of weight, such that mean initial weights of rats in any group did not differ by more than ± 1.0 g. The rats were housed in individual wire-bottom galvanized steel cages that allowed for easy faecal collection and the measurement of food intake. After an acclimatization period of 3 days during which the rats were fed standard stock diet, each group of rats was fed one of the experiment test diets (A–H) and the casein (control) diet (I). The rats had free access to the diets and tap water for 28 days. The temperature of the laboratory was at 28 ± 1 °C throughout the period of the experiment, with alternate periods of light and dark of 12 h. Individual rat body weight, feed intake and feed waste were measured and recorded daily and used in calculating the average 28 days weight gain or loss and

Table 1
Composition of experimental, casein and protein-free diets

Diet[a]	Components (g/100 g)						
	Seed flour	Casein	Corn oil	Salt mixture[b]	Vitamin mixture[c]	Cellulose	Cassava starch
A	30.5	–	8.0	5.0	1.0	1.0	54.5
B	28.4	–	8.0	5.0	1.0	1.0	56.6
C	27.6	–	8.0	5.0	1.0	1.0	57.4
D	27.0	–	8.0	5.0	1.0	1.0	58.0
E	26.6	–	8.0	5.0	1.0	1.0	58.4
F	25.3	–	8.0	5.0	1.0	1.0	59.7
G	25.0	–	8.0	5.0	1.0	1.0	60.0
H	24.8	–	8.0	5.0	1.0	1.0	60.2
I	–	12.5	8.0	5.0	1.0	1.0	72.5
J	–	–	8.0	5.0	1.0	1.0	85.0

[a] Diets provided 10% crude protein; prepared using defatted flours from fluted pumpkin seeds which were fermented for 0 day (A), 1 day (B), 2 days (C), 3 days (D), 4 days (E), 5 days (F), 6 days (G), 7 days (H); I = casein; J = protein-free diet.

[b] Salt mixture (composition per 100 g); calcium (0.6 g); chloride (0.5 g); copper (1.0 mg); iodine (0.03 mg); iron (10.0 mg); magnesium (0.2 g); manganese (7.5 mg); phosphorus (0.5 g); potassium (0.5 g), sodium (0.5 g); zinc (1.8 mg).

[c] Vitamin mixture (composition per 100 g): vitamin A (700 i.u); vitamin D (30 i.u); vitamin E (6 i.u.); vitamin K (0.29 mg). Thiamine hydrochloride (0.6 mg); niacin (1.0 mg); panthothenic acid (1.2 mg); cyanocobalamin, B_{12} (0.5 μg).

protein intake per rat for each group. Protein efficiency ratio (PER) was calculated using standard recommended equations (Pellet and Young, 1980; Jood & Singh, 2001) as:

$$\text{PER} = \frac{\text{Gain (or los)in body weight ('g)}}{\text{Protein consumed (g)}}.$$

$$\text{Corrected PER} = \text{PER} \times \frac{2.50}{\text{Determined PER for casein}}.$$

2.7. Net protein ratio

Net protein ratio (NPR) determination was done on the 10th day of the PER study and lasted for 4 days. In addition to the experimental diets, a control group of rats, matched with the test rats with respect to weight, was fed a protein-free (basal) diet (sample J). Daily records on the weight gain or loss, food and protein intakes were taken and used in calculating NPR by means of the recommended equation (Pellet and Young, 1980; Jood & Singh, 2001):

$$\text{NPR} = \frac{\text{Weight gain of test group} + \text{weight loss of protein-free group}}{\text{Protein consumed by test group}}$$

2.8. Apparent and true digestibilities

The digestibility study was started on the 14th day of the PER study and lasted for 7 days. Carmine red was added to the diets to serve as a marker. Faecal collection began on the appearance of marked (red) faeces. The rats were re-fed the dye-free diet at the end of the 7th day and marked faeces were collected for two more days. Feed intake for the 7-days experimental period was noted and used in calculating protein intake. Fecal output of each rat was collected separately, dried, ground and used for the determination of faecal protein by the Kjeldahl method (AOAC, 1984). The following equations, recommended by Pellet and Young (1980) and Jood and Singh (2001), were used to calculate apparent digestibility (AD) and true digestibility (TD):

$$\text{AD } (\%) = \frac{1 - F}{1} \times 100,$$

$$\text{TD } (\%) = \frac{1 - (F - F_K)}{1} \times 100,$$

where I = Protein intake of rats fed test diet F = Protein excreted in faeces of rats fed test diet F_K = Protein excreted in faeces of rats fed protein-free diet.

2.9. Statistical analyses

All experiments were conducted in triplicate. The means ± standard deviations of three values were calculated. Data were subjected to analysis of variance. If a significant F test was noted, means were separated using a least significant difference test (Wahua, 1999). Significance was accepted at the 0.05 level of probability.

3. Results and discussion

The results of the proximate composition of unfermented and fermented fluted pumpkin seeds are presented in Table 2. The values obtained for crude protein (26.6%) and fat (47.5%) show that fluted pumpkin seed is a good plant protein and oil source. Comparatively lower crude protein values (10.9–24.2%) have been reported for unfermented samples of some commonly consumed oilseeds, legumes and other edible plant seeds used as soup condiments in Nigeria (Ene-Obong & Carnovale, 1992; Giami, Okonkwo, & Akusu, 1994; Giami & Wachukwu, 1997), suggesting that fluted

Table 2
Proximate composition of fermented fluted pumpkin seeds[a]

Fermentation period (day)	Moisture (%)	Crude protein ($N \times 5.30$) (%)	Ether extract (%)	Total ash (%)	Crude fibre (%)	Carbohydrate (by difference) (%)
0 (unfermented)	4.5 ± 0.4	26.6 ± 0.3	47.5 ± 0.5	4.7 ± 0.1	3.1 ± 0.3	13.6 ± 0.5
1	4.8 ± 0.3	26.8 ± 0.7	47.2 ± 0.4	4.8 ± 0.3	3.0 ± 0.1	13.4 ± 0.4
2	4.9 ± 0.1	27.4 ± 0.3	47.1 ± 0.6	4.8 ± 0.1	2.9 ± 0.2	12.9 ± 0.2
3	5.0 ± 0.2	28.8 ± 0.6	45.7 ± 0.8	4.9 ± 0.2	2.8 ± 0.3	12.8 ± 0.3
4	5.4 ± 0.3	28.9 ± 0.4	45.3 ± 0.7	5.0 ± 0.1	2.7 ± 0.2	12.7 ± 0.4
5	5.6 ± 0.4	29.6 ± 0.6	45.0 ± 0.3	5.2 ± 0.3	2.6 ± 0.1	12.0 ± 0.3
6	6.9 ± 0.5	29.8 ± 0.3	43.8 ± 0.1	5.3 ± 0.2	2.3 ± 0.2	11.9 ± 0.6
7	7.2 ± 0.2	30.1 ± 0.8	43.5 ± 0.2	5.4 ± 0.4	2.3 ± 0.2	11.5 ± 0.4
LSD[b] ($p = 0.05$)	0.82	2.62	1.60	0.95	0.50	2.15

[a] Mean ± standard deviation of triplicate determinations.
[b] LSD: differences of two means between samples exceeding this value are significant.

pumpkin seed may be a useful source of protein in the diet of people in tropical countries where this crop is grown. The crude protein content of fermented pumpkin seeds increased by 13.2% and the carbohydrate content decreased by 15.4% during a 7-days fermentation period. Similar, smeller or greater increases in crude protein, as well as decreases in carbohydrate, of various fermented oilseeds have been reported by several other researchers. Isichei and Achinewhu (1988) observed that the crude protein content of African oil bean increased slightly (by 2.0%) whereas the carbohydrate content decreased by 26.5% during a 6-days fermentation period. An increase of up to 28.3% of crude protein and a decrease, varying between 51.8% and 69.4%, of carbohydrate content have been reported during African locust bean fermentation (Eka, 1980; Odunfa, 1986). These reports have compared unfermented seeds with fermented seeds for a given period and under specified conditions; these periods and conditions have varied considerably between reports, making comparison of data impossible. The higher protein in fermented pumpkin seeds may have been partly contributed by the microorganisms involved in the fermentation of the seeds. The protein content of Bacillus spp, the predominant fermentative microorganisms of fluted pumpkin seeds (Barber et al., 1989), have been reported to be high, constituting about 63% of their biomass (Odunfa, 1986).

The NPN (expressed as % of TN) content of the unfermented seed was observed to be 7.37% (Table 3). This value falls within the range of values (6.1–8.7% of TN) reported for NPN contents in twelve unfermented winged bean accessions (Dench, 1982). Fermentation of fluted pumpkin seed for more than 5 days resulted in rapid and significant depletion of PN and increase of NPN, indicating that it may be necessary to consider 5 days as the minimum fermentation period for optimal improvement in the protein content of pumpkin seeds. The increase in crude protein of fermented pumpkin seeds observed in this study may be due, in part, to an increase in NPN.

Albumin and globulin are the major seed proteins of fluted pumpkin seed and they constitute about 59% of the total protein of the unfermented seed (Table 4).

Table 3
Nitrogenous constituents of fermented fluted pumpkin seeds[a]

Fermentation period (day)	Nitrogenous constituents (g/100 g seed flour)			Non-protein nitrogen (% TN)
	Total nitrogen	Protein nitrogen	Non-protein nitrogen	
0 (unfermented)	5.02 ± 0.11	4.65 ± 0.12	0.37 ± 0.03	7.37
1	5.06 ± 0.13	4.62 ± 0.17	0.44 ± 0.03	8.70
2	5.17 ± 0.18	4.61 ± 0.16	0.56 ± 0.02	10.83
3	5.43 ± 0.15	4.60 ± 0.16	0.83 ± 0.01	15.3
4	5.45 ± 0.14	4.59 ± 0.11	0.86 ± 0.02	15.8
5	5.58 ± 0.15	4.58 ± 0.10	1.00 ± 0.06	17.9
6	5.62 ± 0.16	4.11 ± 0.13	1.51 ± 0.05	26.9
7	5.68 ± 0.19	3.98 ± 0.12	1.70 ± 0.05	29.9
LSD[b] ($p = 0.05$)	0.25	0.15	0.38	3.50

[a] Mean ± standard deviation of triplicate determinations.
[b] LSD: differences of two means between samples exceeding this value are significant.

Table 4
Changes in the levels of the seed proteins of fermented fluted pumpkin seeds[a]

Fermentation period	pH	Protein fraction[b](g/100 g of powder)				
		Albumin	Globulin	Prolamine	Glutelin	Total extractable protein (%)
0 (unfermented)	5.5	8.51 ± 0.02	7.08 ± 0.00	3.70 ± 0.01	6.50 ± 0.08	25.8
1	5.2	8.87 ± 0.07	7.90 ± 0.05	2.92 ± 0.03	6.62 ± 0.01	26.3
2	4.8	8.93 ± 0.04	8.00 ± 0.02	2.86 ± 0.01	6.94 ± 0.01	26.7
3	4.5	9.02 ± 0.06	8.06 ± 0.06	2.71 ± 0.08	7.340.04	27.1
4	4.5	9.06 ± 0.07	8.10 ± 0.05	1.68 ± 0.04	8.39 ± 0.02	27.2
5	4.3	9.76 ± 0.06	8.58 ± 0.04	1.19 ± 0.04	8.47 ± 0.05	28.0
6	4.2	7.76 ± 0.04	6.43 ± 0.05	1.18 ± 0.01	10.9 ± 0.07	26.2
7	4.1	7.74 ± 0.03	6.42 ± 0.03	1.17 ± 0.02	11.1 ± 0.06	24.4
LSD[c] ($p = 0.05$)	–	0.50	0.80	0.75	1.00	1.20

[a] Mean ± standard deviation of triplicate determinations.
[b] Estimated by the biuret method.
[c] LSD: differences of two means between samples exceeding this value are significant.

Previous researchers (Shastry & John, 1991) have reported albumin and globulin to be the major storage plant proteins, varying between 50% and 75% of the total seed protein. The albumin and globulin fractions increased during fermentation, reaching their maximum values (34.9% and 30.6% of total extractable protein, respectively), on the 5th day, but declined thereafter. The albumin and globulin protein fractions have been reported to have higher levels of the amino acids lysine (Johnson & Lay, 1974). Thus, the nutritional value of fluted pumpkin seeds, which have been found by Longe et al. (1983) to be deficient in lysine, would be expected to increase due to the increase in the albumin and globulin fractions following 5-days fermentation.

Fermentation increased protein digestibility (in vitro) but decreased polyphenol and phytic acid contents of fluted pumpkin seeds (Table 5). Fermentation for more than 5 days did not bring about further significant improvement in protein digestibility or reduction in the levels of these antinutrients. Protein digestibility of legumes and cereals has been reported to increase as a result of fermentation (Chavan et al., 1988; Taylor & Taylor, 2002). Taylor and Taylor (2002) proposed that, during fermentation, insoluble proteins (prolamine and glutelin) undergo structural changes which make them more accessible to pepsin attack, rather than being broken down into smaller sub-units. These changes are likely to have a marked effect on the digestibility of the seed protein and may be partly responsible for the increased protein digestibility observed in this study in fermented fluted pumpkin seeds. The digestibility of protein in fluted pumpkin seed fermented for 5 days, assessed by the in vitro method in this study, was about 15.8% lower than the true digestibility (TD) of the seed protein, determined, for the same sample, by animal feeding experiments. Michetll and Grundel (1986) reported that assessing the digestibility of a protein using in vitro methods tended to underestimate the protein quality. However, it has been shown (Ruales & Nair, 1994) to give a good relative estimate of protein quality and can be used to screen raw materials and to evaluate the effects of various food processing methods on protein quality. A significant advantage of the *in-vitro* method is that it is cheaper and takes a shorter time to predict the digestibility of the proteins in the sample, unlike digestibility measured by animal feeding which is expensive and time-consuming.

The level of phytic acid in the seeds was reduced by 50.0% during the 7-days fermentation period. It has been suggested that the loss of phytic acid during fermentation might be due to the action of fermenting microorganisms which hydrolyze phytate into inositol and orthophosphate (Sandberg & Andlid, 2002). The low pH of the fermented pumpkin seeds may have provided a favourable condition for phytase activity, in agreement with the views of Harland and Harland (1980). Phytate - degrading enzymes have been detected

Table 5
In vitro protein digestibility, polyphenol and phtyic acid contents of fermented fluted pumpkin seeds[a]

Fermentation period (day)	In vitro protein digestibility (%)	Polyphenol (mg/100 g)	Phytic acid (mg/100 g)
0 (unfermented)	582 ± 0.8	42.6 ± 0.2	299 ± 1.2
1	68.4 ± 0.7	36.1 ± 0.1	169 ± 0.8
2	68.8 ± 0.6	35.9 ± 0.2	165 ± 0.6
3	69.6 ± 0.4	35.6 ± 0.3	163 ± 0.6
4	69.8 ± 0.5	35.3 ± 0.1	161 ± 0.7
5	78.2 ± 0.2	26.8 ± 0.5	131 ± 0.4
6	69.5 ± 0.5	25.6 ± 0.4	127 ± 0.6
7	68.7 ± 0.4	21.3 ± 0.2	121 ± 0.3
LSD[b] ($P = 0.05$)	7.5	4.5	25.0

[a] Mean ± standard deviation of triplicate determinations.
[b] LSD: differences of two means between samples exceeding this value are significant.

Table 6
Protein qualities[a] of experimental diets containing defatted flours from unfermented and fermented fluted pumpkin seeds fed to rats

Diet[b]	Weight gain/loss (g)	Protein intake (g)	Protein efficiency ratio		Net protein ratio	Apparent digestibility (%)	True digestibility (%)
			Actual	Adjusted			
A (unfermented)	−3.10 ± 0.02	1.86 ± 0.12	−1.67 ± 0.03	−1.69 ± 0.02	1.38 ± 0.01	45.3 ± 0.83	61.8 ± 1.30
B	12.07 ± 0.04	15.9 ± 0.12	0.76 ± 0.05	0.77 ± 0.03	1.52 ± 0.03	56.7 ± 1.01	68.1 ± 1.00
C	14.77 ± 0.13	16.4 ± 0.20	0.90 ± 0.01	0.91 ± 0.02	2.30 ± 0.04	58.4 ± 1.20	70.6 ± 1.14
D	24.14 ± 0.21	16.65 ± 0.57	1.45 ± 0.13	1.46 ± 0.04	2.42 ± 0.06	60.7 ± 1.32	72. 2 ± 1.16
E	31.47 ± 0.63	20.8 ± 0.72	1.51 ± 0.15	1.53 ± 0.06	2.68 ± 0.07	61.1 ± 1.03	78.5 ± 1.24
F	52.38 ± 1.00	21.82 ± 0.81	2.40 ± 0.12	2.42 ± 0.10	4.13 ± 0.05	88.7 ± 1.34	92.9 ± 1.08
G	29.36 ± 0.54	18.1 ± 0.44	1.62 ± 0.09	1.64 ± 0.05	2.70 ± 0.01	62.5 ± 0.82	76.9 ± 1.02
H	18.59 ± 0.47	13.5 ± 0.06	1.38 ± 0.01	1.39 ± 0.02	2.45 ± 0.06	59.7 ± 0.68	71.3 ± 0.72
I	54.86 ± 1.00	22.1 ± 0.41	2.48 ± 0.01	2.50 ± 0.06	4.45 ± 0.08	86.6 ± 0.63	94.7 ± 1.50
LSD[c] ($p = 0.05$)	11.50	5.00	0.50	0.50	0.82	10.3	14.1

[a] Mean ± standard deviation of ten rats per group.
[b] Prepared from fluted pumpkin seeds fermented for 0 day (A), 1 day (B) 2 days (C), 3 days (D), 4 days (E), 5 days (F), 6 days (G), 7 days (H); I = casein (control).
[c] LSD: differences of two means between samples exceeding this value are significant.

in various bacterial genera, such as *Bacillus* (Kerovuo, Ruovinen, & Hatzack, 2000) and *Pseudomonas* (Richardson & Hadobas, 1997). These bacterial genera have been isolated from fermenting fluted pumpkin seeds (Barber et al., 1989).

Rats fed diets formulated with unfermented samples of fluted pumpkin seed flour lost weight and the diet resulted in poor protein quality indices, such as negative values for PER, and low values for NPR and TD (Table 6). Feeding studies by other researchers showed growth depression in experimental animals for diets containing unfermented fluted pumpkin seeds (Longe et al., 1983; Achinewhu & Isichei, 1990). These reports attributed this result to toxic components which tended to impair protein utilization, thereby reducing the nutritional value of the seed proteins. The weight gain of rats fed diets formulated with fluted pumpkin seeds fermented for 5 days was significantly ($p < 0.05$) higher than those of rats fed diets formulated with seeds fermented for shorter or longer periods of time. The values obtained for PER, NPR and TD of rat diets containing fluted pumpkin seeds fermented for 5 days were similar to those of casein, indicating that the nutritional quality of fluted pumpkin seeds improved following a 5-days fermentation period.

This study has shown that fermentation increased crude protein, NPN and glutelin protein fractions and decreased the PN, polyphenol and phytic acid contents of fluted pumpkin seeds. Also, the diet formulated with pumpkin seeds fermented for 5 days was nutritionally comparable to a diet based on casein.

References

Achinewhu, S. C. (1986). Some biochemical and nutritional changes during the fermentation of fluted pumpkin (*Telfairia occidentalis*). *Plant Foods for Human Nutrition, 36*, 97–106.

Achinewhu, S. C. (1987). Protein quality evaluation of weaning food mixtures from indigenous fermented foods. *Nigerian Journal of Nutritional Sciences, 8*, 23–31.

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

AOAC (1984). Official Methods of Analysis, 14th Association of official Analytical Chemists, Washington, DC.

Bajaj, S., Michelson, O., Lillevik, H. A., Baker, L. R., Bergen, W. G., & Gill, J. L. (1971). Prediction of protein efficiency ratio of peas from their albumin content. *Crop Science, 11*, 813–815.

Banigo, E. B., & Akpapunam, M. A. (1987). Physico–Chemical and nutritional evaluation of protein-enriched fermented maize flour. *Nigerian Food Journal, 5*, 30–36.

Barber, L. I., Ibiama, E. A., & Achinewhu, S. C. (1989). Microorganisms associated fermented fluted pumpkin seeds (*Telfairia occidentalis*). *International Journal of Food Science and Technology, 24*, 189–193.

Biehl, B., Wewetzer, C., & Passern, D. (1982). Vacuolar (storage) proteins of cocoa seeds and their degradation during germination and fermentation. *Journal of the Science of Food and Agriculture, 33*, 1291–1304.

Chavan, U. D., Chavan, J. K., & Kadam, S. S. (1988). Effect of fermentation on soluble proteins and in vitro protein digestibility of sorghum, green gram and sorghum – green gram blends. *Journal of Food Science, 53*, 1574–1575.

Dench, J. E. (1982). Extraction of nitrogenous material from winged bean *Psophocarpus tetragonolobus* (L.) (DC) flour and the preparation and properties of protein isolates. *Journal of the Science of Food and Agriculture, 33*, 173–184.

Dhankher, N., & Chauhan, B. M. (1987). Effect of temperature and fermentation time on phytic acid and polyphenol content of rabidi – a fermented pearl millet food. *Journal of Food Science, 52*, 828–829.

Eka, O. U. (1980). Effect of fermentation on the nutrient status of locust bean. *Food chemistry, 5*, 303–308.

El-Khalifa, A. O., & El-Tinay, A. H. (1994). Effect of fermentation on protein fractions and tannin content of low and high tannin cultivars of sorghum. *Food Chemistry, 49*, 265–269.

Ene-Obong, H. N., & Carnovale, E. (1992). Nigerian soup condiments: Traditional processing and potential as dietary fibre sources. *Food Chemistry, 43*, 29–34.

Frais, F. (1972). *Practical biochemistry, an introductory course*. London: Butterworth Publishers, pp. 90–91.

Giami, S. Y., & Bekebain, D. A. (1992). Proximate composition and functional properties of raw and processed full-fat fluted pumpkin (*Telfairia occidentalis*) seed flour. *Journal of the Science of Food and Agriculture, 59*, 321–325.

Giami, S. Y., Okonkwo, V. I., & Akusu, M. O. (1994). Chemical composition and functional properties of raw, heat-treated and partially proteolysed wild mango (*Irvingia gabonensis*) seed flour. *Food Chemistry, 49*, 237–243.

Giami, S. Y., & Wachukwu, O. C. (1997). Composition and functional properties of unprocessed and locally processed seeds from three underutilized food sources in Nigeria. *Plant Foods for Human Nutrition, 50*, 27–36.

Giami, S. Y., & Isichei, I. (1999). Preparation and properties of flours and protein concentrates form raw, fermented and germinated fluted pumpkin (*Telfairia occidentalis* Hook) seeds. *Plant Foods for Human Nutrition, 54*, 67–77.

Giami, S. Y. (2002). Chemical composition and nutritional attributes of selected newly develop lines of soybean (*Glycine max* L. Merr). *Journal of the Science of Food and Agriculture, 82*, 1735–1739.

Harland, B. F., & Harland, J. (1980). Fermentative reduction of phytate in rye, white and whole wheat bread. *Cereal Chemistry, 57*, 226–229.

Isichei, M. O., & Achinewhu, S. C (1988). The nutritive value of African oil bean seeds (*Pentaclethra macrophylla*). *Food Chemistry, 30*, 83–92.

Johnson, V. A., & Lay, C. L. (1974). Genetic improvement of plant proteins. *Journal of Agricultural and Food Chemistry, 22*, 558–566.

Jood, S., & Singh, M. (2001). Amino acid composition and biological evaluation of the protein quality of high lysine barley genotypes. *Plant Foods for Human Nutrition, 56*, 145–155.

Kerovuo, J., Ruovinen, J., & Hatzack, F. (2000). Hydrolysis of phytic acid by *Bacillus phytase*. *Biochemistry Journal, 352*, 623–628.

Longe, O. G., Farinu, G. O., & Fetuga, B. L. (1983). Nutritional value of the fluted pumpkin (*Telfairia occidentalis*). *Journal of Agricultural and Food chemistry, 31*, 989–992.

Mertz, E. T., Veron, O. A., & bates, L. S. (1965). Growth of rats fed on opaque – 2 maize. *Science, 148*, 1741–1743.

Michetll, G. V., & Grundel, E. (1986). Nutritional value of proteins in powdered infant formula: In vitro and in vitro methods. *Journal of Agricultural and Food chemistry, 34*, 650–653.

Nwokolo, E., & Sim, J. S. (1987). Nutritional assessment of defatted oil meals of melon (*Colocynthis citrullus*) and fluted pumpkin (*Telfairia occidentalis*) by chick assay. *Journal of the Science of Food and Agriculture, 38*, 237–246.

Odunfa, S. A. (1986). Dawadawa. In N. R. Reddy, M. D. Pierson, & D. K. Salunkhe (Eds.), *Legume based fermented foods* (pp. 173–189). Boca Raton, FL: CRC press.

Osborne, D. R., & Voogt, P. (1978). *The analysis of nutrients in foods*. New York: Academic press, pp. 120–121.

Pellet, P. L., & Young, V. R. (1980). Nutritional evaluation of protein foods. Food and Nutrition Bulletin, Supplement No. 4, United Nations University, World Hunger Programme: Japan.

Richardson, A. E, & Hadobas, P. A. (1997). Soil isolates of *Pseudomonas* spp. that utilize inositol phosphates. *Canadian Journal of Microbiology, 43*, 509–516.

Ruales, J., & Nair, B. M. (1994). Effect of processing on in vitro digestibility of protein and starch in quinoa seeds. *International Journal of Food Science and Technology, 29*, 449–456.

Sandberg, A. S., & Andlid, T. (2002). Phytogenic and microbial phytases in human nutrition. *International Journal of Food Science and Technology, 37*, 823–833.

Saunders, R. M., Connor, M. A., Booth, A. N., Bickoff, E. M., & Kohler, G. O. (1973). Measurement of digestibility of alfalfa protein concentrates by in vivo and in vitro methods. *Journal of Nutrition, 103*, 530–535.

Shastry, M., & John, E. (1991). Biochemical changes and in vitro protein digestibility of the endosperm of germinating *Dolichos lablab*. *Journal of the Science of Food and Agriculture, 55*, 529–538.

Taylor, J., & Taylor, J. R. N. (2002). Alleviation of the adverse effect of cooking on sorghum protein digestibility through fermentation in traditional African porridges. *International Journal of Food Science and Technology, 37*, 129–137.

Wahua, T. A. T. (1999). *Applied statistics for scientific studies*. Aba Nigeria: African-link Press.

Wheeler, E. L., & Ferrel, R. E (1971). A method for phytic acid determination in wheat and wheat fractions. *Cereal Chemistry, 48*, 312–316.

Wilson, M. F., & Blunden, C. A. (1983). Changes in the levels of polyphenols in three pear varieties during bud development. *Journal of the Science of Food and Agriculture, 34*, 973–978.