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Effect of Bioprocess on Nutritional Quality and Chemical Properties of Bambara Groundnut (Vigna Subterranean (L) Verdc.) Flour

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Abstract
The effect of bioprocess on the nutritional quality of bambara groundnut (*Vigna subterranean* (L) *Verdc.*) flour was studied and *in-vitro* protein digestibility of the raw (non-bioprocessed) flour was found to increase from 70.74 to 89.70% after fermentation while anti-nutritional factors was found to decrease from 4.72, 870.30, 1470.15, 1.85 and 8.40 mg/100gm to 2.08, 383.70, 1023.10, 0.55 and 3.30 mg/100 gm for tannin, polyphenol, phytate, oxalate and trypsin inhibition activity (TIA) respectively. The results was evident that the antinutrient concentration in bambara groundnut can be eliminated or reduced to tolerable level through the bioprocess of fermentation, thus increase protein digestibility. The crude protein content of raw bambara seeds was found to be 20.80% and slightly decreased after fermentation (19.70%). The results showed that bioprocess had no significant effect on protein content. Crude fat content significantly ($p < 0.05$) increased from 6.80 to 8.79% after fermentation while carbohydrate content was found to decrease from 57.20 to 52.25% and this was attributed to the possible breakdown and utilization of the sugars by the fermenting organisms as a ready source of energy. Fiber content significantly ($p > 0.05$) decreased from 6.60 to 3.98%; and this can also be attributed to the partial solubilization of cellulose and hemicellulosic type of material by microbial enzymes. Ash content was found to decrease from 3.45 to 3.18%; while dry matter slightly increased from 93.80 to 94.50%. Results obtained for gross energy showed that bioprocess significantly ($p < 0.05$) increased the energy value to 425.10 kCal/100 gm. The calculated metabolizable energy values, which ranged between 368.10 and 425.10 kCal/100 gm showed that bambara groundnut have energy concentrations favorably comparable to cereals.
Keywords: Bioprocess, Bambara Groundnut, Anti-nutrients, Protein Digestibility

INTRODUCTION

Bambara groundnut (*Vigna subterranean* (L) *Verdc.*) is a highly nutritive but underutilized grain legume indigenous to West and Central Africa which can be used to solve food security issues in these regions. Hence, it contains anti-nutritional factors which can cause detrimental effects to humans and animal growth and performance by impairing intake, uptake, or utilization of other foods and feed components or by causing discomfort and stress to humans and animals. These anti-nutritional factors mainly occur in pulses and grain legumes and foods and feed material prepared from grain legumes and pulses (Friedman, 2001).

However, fermentation is an age-old traditional method of processing grain legumes, seeds, and nuts in Africa. Grain legumes are fermented in African countries for a number of reasons. These include modifying the physical, nutritional, and sensory status of the raw materials (Barimalaa and Anoghalu, 1997), to yield products of overall improved quality; and to reduce substances such as trypsin inhibitors, which reduce protein digestibility, and low-molecular weight carbohydrate fractions, which cause flatulence on ingestion. The present research studies the effect of bioprocess on *in-vitro* protein digestibility and anti-nutrients as well as the chemical properties of the bioprocessed bambara groundnut flour in comparism to the non-bioprocessed bambara groundnut flour.

MATERIALS AND METHODS

Collection and Preparation of Samples: Bambara groundnut was purchased from Ogbete Main Market, Enugu State of Nigeria. The bamabara groundnuts were carefully cleaned and freed of all extraneous materials as well as damaged nuts prior to use. The nuts was washed twice with ordinary water, rinsed with distilled water, and cooked to softness as a pretreatment measure and to eliminate existing microflora. Pure cultures of freeze dried *Lactobacillus plantarum* [NRRL B-4306] and *Lactobacillus fermentum* [NRRL B-1932] preserved in a dormant state by drying a heavy suspension of cells in sterile bovine serum was obtained from Agricultural Research Services Culture Collection, Bacterial Foodborne Pathogens and Mycology Research Unit; National Center for Agricultural Utilization Research of the United States Department of Agriculture, Peoria Illinois USA. The freeze dried cells was brought to active state by growing in 25 ml sterile M.R.S. broth, and incubated in CO2 enriched jars for 24 h and centrifuged at 3600-x g for 15 min. The recovered cells were rinsed using 10 ml sterile

distilled water and spine twice at 3600-x g for 15 min. After this, a 9 ml suspension of the cells was made using sterile distilled water. The suspensions was serially diluted and plated out on plate count agar using the pour plate method. After 24 h incubation period in CO2 enriched jars, the colonies on each plate of dilution factor was counted and the plate with approximately 10^6 cfu/ ml was noted and used at every inoculation of the fermentation process.

Fermentation: Twenty (20) kg of bambara groundnuts were cooked to softness and was rinsed with distilled water and poured into a basin. Ten (10) ml inoculum suspension of *Lactobacillus plantarum* [NRRL B-4306] and *Lactobacillus fermentum* [NRRL B-1932] containing approximately 10^6 cfu/ml was then inoculated aseptically into the 10kg of bambara groundnuts used for this study and 15 liters of distilled water added. The basin was covered completely and allowed to stand on the laboratory bench for three days at room temperature for the nuts to ferment. The fermentation was carried out without stirring, in accordance with the usual household practice. Thus, the uninoculated 10 kg of the nuts were used as control. The uninoculated (non-bioprocessed) sample was drained of water and the nuts spread on a tray and dried in a cabinet dryer at 60^{o}C for 14 h; hence, the same process was repeated for the inoculated sample after the fermentation period. To obtain the whole bambara groundnut flour; the samples were finely milled using commercial attrition grinder and sieved 3 times using a laboratory test sieve (Sethi Standard Test Sieve 100 BSS). The flour was stored in an airtight nylon bags at 4°C until it was used for experiments.

Determination of Effect of Bioprocess on *In-Vitro* Protein Digestibility: *In-vitro* protein digestibility of bioprocessed and non-bioprocessed samples was measured according to the method of Maliwal (1983). A known weight of the sample containing 16 mg nitrogen was taken in triplicate and digested with 1 mg pepsin in 15 ml of 0.1 M HCl at 37ºC for 2 h. The reaction was stopped by the addition of 15 ml of 10% trichloroacetic acid (TCA). The mixture was then centrifuged at 630 gm for 5 min and was filtered quantitatively through Whatman No. 1 filter paper. The TCA soluble fraction was assayed for nitrogen using the micro-Kjeldahl method (AOAC, 1990). Digestibility was obtained by using the following equation:

$$\text{Protein digestibility (\%)} = \frac{\text{N in supernatant - N in blank}}{\text{N in sample}} \text{ X } 100$$

Determination of Effect of Bioprocess on Antinutrients (Tannin, Phytate, Oxalate, Polyphenols and Trypsin Inhibition Activity)

Tannin content determination: Quantitative determination of tannins was carried out using the modified vanillin-HCl method according to Price *et al*. (1978). A 200 mg sample was extracted with 10 ml 1% (v/v) conc. HCl in methanol for 20 min in capped rotating test tubes. Vanillin reagent (0.5%, 5 ml) was added to extract (1 ml) and the absorbance of the color developed after 20 min at 30°C was read at 500 nm. Correction for interference light natural pigments in the sample was achieved by subjecting the extract to conditions of the reaction, but without vanillin reagent. A standard curve was prepared expressing the results as catechin equivalents, i.e amount of catechin (mg per ml) which gives a color intensity equivalent to that given by tannins after correcting for blank.

Phytic acid content determination: Phytic acid was extracted from each 3 g of samples with 3% trichloro-acetic acid by shaking at room temperature followed by high speed centrifugation as described by Wheeler and Ferrel (1971). The phytic acid in the supernatant was precipitated as ferric phytate and iron in the sample estimated. Phytate-phosphorus (phytate-P) was calculated from the iron results assuming a 4:6 iron: phosphorus molecular ratio. The phytic acid was estimated by multiplying the amount of phytate-phosphorus by the factor 3.55 based on the empirical formula C6P6O24H18.

Oxalate content determination: Oxalate content was determined by AOAC (1990) method. One gram of the sample was weighed into 100 ml conical flask while 75 ml of 3 M H2SO4 was added and the solution carefully stirred intermittently with a magnetic stirrer for about 1 h and then filtered using whatman No.1 filter paper. The sample filtrate (extract) (25 ml) was collected and titrated against hot (80 - 90°C) 0.1 N KMnO4 solution to the point when a faint pink color appeared that persisted for at least 30 s. The concentration of oxalate in each sample was obtained from the calculation: 1 ml 0.1 permanganate = 0.006303 g oxalate.

Total polyphenols determination: Total polyphenols was determined by spectrophotometric method described by Price and Butler (1977). About 60 mg of the sample was shaken manually for 60 s with 3 ml of methanol in a test tube. The mixture was then filtered and the tube quickly rinsed with additional 3.0 ml of methanol and the contents poured at once into a funnel. The filtrate was mixed with 50 ml of water and analyzed within an hour. Three ml of 0.1 M FeCl3 in 0.1 N HCl was added to 1.0 ml of filtrate, followed immediately by timed addition of 3ml of 0.008 M K3Fe(CN)6. The absorbance was read at 720 nm after 10 min using a spectrophotometer. Tannic acid was used to prepare a standard curve following the above procedure.

Trypsin inhibition activity (TIA): The trypsin inhibition activity was assayed in terms of the extent to which an extract of the defatted flour inhibited the action of bovine trypsin (EC 3.4.21.4) on the substrate benzoyl-DL-arginine-p-nitrianilide (BAPNA) hydrochloric (Kakade *et al*., 1974). The samples (1g each) were extracted

continuously at ambient temperature for 3 h with 50 mL, 10 mM NaOH using a mechanical shaker. The pH of the resulting slurry was adjusted to 9.4 - 9.6 with 1 M NaOH. After extraction, the suspension was shaken and diluted with distilled water such that 1 cm^3 of the extract produced trypsin inhibition of 40 - 60% at 37°C. The respective dilutions were noted. Consequently, TIA was calculated in terms of mg pure trypsin (Sigma type lll, lot 20H0868).

$$TIA = \frac{2.632DA}{S} \text{ mg pure trypsin inhibited g}^{-1} \text{ sample}$$

Where D is the dilution factor, A is the change in absorbance at 410 mm due to trypsin inhibition per cm^3 diluted sample extract and S is the weight of the sample.

Determination of Effect of Bioprocess on Chemical Properties: The samples were analyzed for crude protein, crude fat, crude fiber and total ash by following their respective procedures described in AACC (2000). Crude protein (N × 6.25) was determined by the Kjeldahl method. Crude fiber was determined after digesting a known weight of fat-free sample in refluxing 1.25% sulfuric acid and 1.25% sodium hydroxide. Ash was determined by incineration (550°C) of known weights of the samples in a muffle furnace. Crude fat was determined by exhaustively extracting a known weight of sample in petroleum ether (boiling point, 40 to 60°C) in a soxhlet extractor. The carbohydrate content was determined by subtracting the total crude protein, crude fiber, ash and fat from the total dry weight (100g) of the sample differences.

The gross energy was determined with a ballistic bomb calorimeter and the caloric value estimation was done according to Antia *et al.* (2006) by summing the multiplied values for crude protein, oil, and carbohydrate by their respective factors.

Statistical Analysis: Data generated from this study were represented as mean ± standard deviation and statistically analyzed using one-way analysis of variance and mean separation was done by Duncan's new multiple range and Paired T-tests at 95 % level.

RESULTS

The result of *in-vitro* protein digestibility of the raw bambara groundnut was found to be 70.74%. Cooking and fermentation of the nut significantly ($p < 0.05$) increased the *in-vitro* protein digestibility to 89.70% and the results shown in Table 1.

Table 2 shows the anti-nutritional factors of raw and bioprocessed bambara groundnut flour. Tannin content of the flour from raw nut was found to be 4.72 mg/100 gm, which was higher than the values obtained in some other studies. . Cooking and fermentation significantly ($p > 0.05$) decreased tannin content to 2.08 mg/100 gm. Polyphenol content of raw bambara nut flour was found to be 870.30 mg/100 gm which was significantly ($p > 0.05$) decreased after cooking and fermentation to 383.70 mg/100 gm. The phytate content of the raw bambara groundnut flour was found to be 1470.15 mg/100 gm. The nut are rich in protein (20.60%), therefore they had high phytate levels. Oxalate content was found to be 1.85 mg/100 gm for raw bambara groundnut flour. Cooking and fermentation of the nut significantly ($p > 0.05$) decreased oxalate content to 0.55 mg/100 gm. Trypsin inhibition activity (TIA) has a content of 8.40 mg/100gm for raw bambara groundnut flour. Thus, cooking and fermentation of the nut significantly ($p > 0.05$) decreased the content of trypsin inhibitor to 3.30 mg/100 gm.

Table 3 presents the chemical composition of the raw and fermented bambara groundnut flour. The protein content of bambara seeds was found to be 20.80%. The protein content of the nut slightly decreased after fermentation (19.70%). The results showed that bioprocessing of the seeds had no significant ($p > 0.05$) effect on protein content. The fat content of bambara seeds was 6.80% which was similar to that reported other researchers and higher than that of Mune *et al*. (2007) for raw bambara seeds. Bioprocessing had significantly ($p < 0.05$) increased fat content of the bambara groundnut after fermentation. The carbohydrate content of bambara groundnut was found to be 57.20% which was similar to that reported by other researchers. The carbohydrate content significantly ($p > 0.05$) decreased after fermentation. Fiber content of raw bambara seeds was 6.60% which varied among other works done by other researchers. The fiber content of bambara groundnut flour significantly ($p > 0.05$) decreased after bioprocessing (3.98%). Ash content was found to be 3.45% which was lower than that obtained by other researchers. The ash content was slightly decreased after fermentation. Dry matter of raw flour was 93.80% which slightly increased after fermentation. The result obtained was higher than that of other researchers, thus, no significant ($p > 0.05$) difference was observed in dry matter after bioprocess. The gross energy content of the bambara groundnut significantly ($p < 0.05$) increased after bioprocess with a maximum value of 425.10 kCal/100 gm.

Table 1: *In Vitro* Protein Digestibility (IVPD) of Raw and Fermented Flour

Analysis	Raw Sample	Fermented Sample
IVPD (%)	70.74±1.0	89.70±0.6

Values are means ± SD (n = 3)

Table 2: Antinutritional Factors (mg/100 gm) of Raw and Fermented Flour

Antinutrients	Raw Sample	Fermented Sample
Tannin	4.72±1.0	2.08±0.2
Polyphenols	870.30±0.3	383.70±0.5
Phytic Acid	1470.15±0.5	1023.10±0.1
Oxalate	1.85±0.8	0.55±0.5
Trypsin Inhibitor	8.40±0.2	3.30±0.4

Values are means ± SD (n = 3)

Table 3: Chemical Composition (%) and Total Energy (kCal/100g) of Raw and Fermented Flour

Parameters	Raw Sample	Fermented Sample
Protein (%)	20.80±0.8	19.70±0.2
Fat (%)	6.80±0.1	8.79±0.5
Fiber (%)	6.60±0.3	3.98±0.1
Carbohydrate (%)	57.20±0.4	52.25±0.7
Ash (%)	3.45±0.2	3.18±0.3
Dry Matter (%)	92.80±0.1	94.50±0.5
Energy (kCal/ 100g)	368.10±1.0	425.10±1.5

Values are means ± SD (n = 3)

DISCUSSION

The increment in protein digestibility after fermentation of the nut observed in this study, is likely due to reduction in antinutrients as a result of cooking and subsequent fermentation. Effective reduction of antinutrients has been reported to improve protein digestibility in legumes (Babiker and El Tinay, 1993). Tannin content of the flour from raw nut was found to be higher than that reported by Abiodun and Adepeju (2011) for bambara groundnut. The significant decreased in cooking and fermentation was observed by Mubarak (2005), Hassan *et al.* (2005) and Abedel Hady *et al.* (2005) for soaking and cooking of mug bean, lubin, maize and lentil seeds respectively.

The significant decreased in polyphenol content of raw bambara nut flour after cooking and fermentation were in agreement with the findings of Yagoup *et al.* (2004) for roselle seeds. The reduction in polyphenols may be due to washing out of soluble polyphenols in water and due to interaction with protein during cooking forming poorly extractable protein phenolic complexes.

There was high phytate content observed in the raw bambara groundnut flour studied. In legumes, phytates are associated with protein bodies (Sulieman *et al*., 2007) and, therefore, phytate levels should increase with increasing protein content. The loss in phytates during cooking and fermentation of bambara nut may be due to leaching of phytate ions into the cooking and fermentation water under the influence of a concentration gradient (difference in chemical potential), which governs the rate of diffusion. Similar results for reduction in phytic acid in soaked bean have been earlier reported (Bishnoi *et al*., 1994). The significant decreased oxalate content after cooking and fermentation of the nut was observed by Ijarotimi and Esho (2009) for roasted bambara groundnut as weaning diet.

There was significant decreased in the content of trypsin inhibitor after cooking and fermentation in the present study. Although no literature was found to support reduction using a bioprocess, a research conducted at the University of Yaounde in Cameroon however showed that treatment of bambara groundnut flour with 60% alcohol, decreases antinutritional factors especially trypsin inhibitor and eliminates flatulence-inducing sugars (Millward and Jackson 2004). Legume consumption has been related to various deleterious effects, such as growth retardation (Martinez *et al*., 1995), lowered digestibility, and absorption of dietary nutrients (Pusztai *et al.*, 1995) and physiological, metabolic, and immunological disturbances (Hajobs *et al.*, 1995). However, it is evident that the anti-nutrient concentration in legumes can be eliminated or reduced to tolerable level through the bioprocess of fermentation.

The protein content of bambara seeds was found to be similar to that reported by Abdulsalami and Sheriff (2010) but higher than that reported by Okonkwo and Opara (2010). However, an increment in protein content after soaking was observed by Hassan *et al.* (2005) for Lupin seeds and explained that increment to quantitative reduction of the antinutritional factors (tannin and phytic acid) and other water soluble constituents. In this study,

the decrease in protein content after bioprocessing may have resulted from precooking the nut possibly due to solublization of protein by heating that lead to loss of protein in the final product as explained by Deman, (1999) or might be attributed to denaturation of it during drying as reported by Bradbury *et al.* (1984). Similar reduction in protein after cooking was observed by Hamed *et al*. (2008) and Hainida *et al.* (2008) for pumpkin and roselle seeds, respectively.

It was observed in the present study that the fat content of bambara seeds was was similar to that reported by Abdulsalami and Sheriff (2010) and higher than that of Mune *et al*. (2007) for raw bambara seeds. This could be attributed to bioprocessing, which had significantly increased fat content of the bambara groundnut after fermentation. Kazanas and Fields (1981) did not observe any significant changes in the crude fat content of sorghum after lactic acid fermentation for 4 days. Chavan (1988) found a slight increased in crude fat content of sorghum and sorghum plus green gram blend during natural fermentation. The carbohydrate content of bambara groundnut was found to be was similar to that reported by Okonkwo and Opara (2010). The changes observed wer possibly due to leaching of soluble components into cooking (Yagoub and Abdalla, 2007) and fermentation water; and possibly the breakdown and utilization of the sugars by the fermenting organisms as a ready source of energy. Carbohydrates particularly starch and soluble sugars are principal substrates for fermentation with lactics. Hence, significant degradation and a subsequent decrease in starch content are expected to occur during fermentation of legumes.

Fiber content of raw bambara seeds was similar to that obtained by Abdulsalami and Sheriff (2010), and higher than that reported by Mune *et al*. (2007). The significant decreased in the fiber content after bioprocessing could be attributed to the breakdown and actions of the fermenting organisms and can also be attributed to the partial solubilization of cellulose and hemicellulosic type of material by microbial enzymes. Ash content was found to be lower than that obtained by Abdulsalami and Sheriff (2010) and Mune *et al.* (2007) for raw bambara groundnut. The ash content was slightly decreased after fermentation, which agrees with the findings of Abdulsalami and Sheriff (2010) after cooking of bambara groundnut seeds. Dry matter of raw flour was slightly increased after fermentation. The result obtained was higher than that of Okonkwo and Opara (2010), for raw bambara groundnut, thus, no significant difference was observed in dry matter after bioprocess. Chavan (1988) observed a loss of only 2.3% for sorghum during 2 days, 9.7% for green gram, and 9.4% for sorghum plus green gram after 2 days fermentation using 1:3 ratios of solids: water. El-Tinay *et. al.* (1979) observed that the dry matter losses increased from about 6% (1:1, solid: water) to about 16.5% (1:8, solid: water) after 4 days fermentation of corn meal. The calculated metabolizable energy values, which ranged between 368.10 and 425.10 kCal/100 gm showed that bambara groundnut have energy concentrations favorably comparable to cereals.

CONCLUSION

Bambara groundnut is an underutilized crop that has been identified to have the potential to contribute towards improving food security. At present, the commercial potential of this nut remains largely unexploited and this can only be remedied through effective processing. Fermentation is an age long process used to raid foods of unwanted substances. The safety of food fermentation processes is related to several principles. The first is that food substrates overgrown with desirable, edible microorganisms become resistant to invasion by spoilage, toxic or food poisoning microorganisms. Hence, as fermented foods generally have a very good safety record even in villages where the foods are manufactured by people without training in microbiology or chemistry and in unhygienic contaminated environments. It becomes imperative to employ the bioprocess of fermentation as a treatment measure to reduce these anti-nutritional components found in bambara groundnut to a safe level and improve its digestibility. Thus, While bambara groundnut is not a toxic substance, the present study observed that albino rats fed fermented bambara groundnut performed better in terms of all the parameters studied thus indicating more availability of nutrients and removal of anti-nutritional factors.

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