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Effect of Bioprocess on Amino Acid Profile and Mineral Extractability of Bambara Groundnut (*Vigna subterranean* (L) *Verdc.*) Flour

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Abstract

The effect of microbial fermentation on amino acid composition and extractability of minerals in Bambara groundnut flour was studied. The bioprocessed flour was produced using submerged fermentation with bacterial strains of *Lactobacillus plantarum* [NRRL B-4306] and *Lactobacillus fermentum* [NRRL B-1932] obtained from the United States Department of Agriculture (USDA). Analysis of amino acid composition observed a slight increase in all the amino acids while glutamic acid (17.20 - 19.50 mg/100 g), aspartic acid (5.62 - 6.80 mg/100 g) and leucine (7.00 - 8.00 mg/100 g) are the most abundant for non-inoculated and inoculated samples respectively. When compared, the bioprocessed sample was found to meet the WHO/FAO daily dietary recommendation. Calcium content of flour from non-inoculated nuts was 220.30 mg/100 g which decreased to 198.80 mg/100 g after fermentation. HCl-extractability of calcium in non-inoculated sample was found to be 80.50%. Extractable calcium level, after bioprocess significantly increased to 89.90%. HCl-extractability of all other major minerals followed a trend similar to that obtained for calcium. The treatment also significantly increased HCl-extractability of iron ($p < 0.05$) from the control value of 65.28% to 75.80%. HCl-extractability of all other trace minerals followed a trend similar to that obtained for iron. The study suggests that microbial fermentation may offer a simple means to improve the nutritional pattern of this crop, hence, optimize its utilization.

Keywords: Bioprocess, Starter Culture, *Lactobacillus*, Amino Acid, Recommended Daily Intake

INTRODUCTION

Plant foods contribute significantly to human nutrition in Africa, since nutrients of animal origin are either scarce or expensive (Obizoba and Atii, 1991). Grain legumes and nuts are important sources of protein, vitamins and minerals in the African diet. Approximately 20 percent of the protein currently available to man is derived solely from legumes in developing world (Afolabi, 2012). These plant foods are generally subjected to fermentation processing prior to consumption. Fermentation is an age-old traditional method of processing grain legumes, seeds and nuts in Africa. Fermented legumes, seeds and nuts are therefore important component of the African diet. They are generally used either as meat substitutes or as condiments.

Bambara groundnut (*Vigna subterranean* (L)), is a seed whose origin is of West and Central Africa where research and development is ineffective, however, the crop has been identified to have the potential to improve malnutrition and boost food availability. Studies have shown the importance of harnessing the nutritive values of Bambara groundnut as an alternative source of proteins that could alleviate the problem of protein deficiency in developing regions of the world. Several workers have examined the biochemical composition of the nut (Okonkwo and Opara, 2010; Mune *et al.*, 2007).

While many foods become even healthier when fermented due to elimination of antinutrients, improved nutrient bioavailability and increased production or creation of phytonutrients. The combination of increased technical precision in fermentation and greater awareness of the advantages has led to a surge in interest in fermentation-derived food processing which has shown to result in improved amino acid profile of foods.

These amino acids are organic compounds that contain amine (-NH2) and carboxyl (-COOH) functional groups, along with a side chain (R group) which is specific to each amino acid (Nelson and Cox, 2005). Numerous traditional techniques for preparation of diets in Africa and other regions of the world involve a fermentation step, and studies of these methods show nutritional improvements which include higher digestibility and improved amino acid profile compared to foods prepared from unfermented grain (Hassan and Tinay, 1995). In a study, the relative nutritional value (RNV) of maize increased from 65% to 81% when it was germinated and fermentation of the flour made of the germinated maize gave a further increase in RNV to 87% (Lay and Fields, 1981).

Thus, the development of an inexpensive processing method that could both improve the digestibility of protein in Bambara groundnut and increase the protein content and amino acid profile could make it a more desirable ingredient for food or feed applications. These developments and improvement of microbial starters has been a driving force for the transformation of traditional food fermentations in developing countries from an “art” to a science. The present study aims to analyze the effect of starter culture fermentation on the amino acid composition and extractable minerals of flour produced from Bambara groundnut.

MATERIALS AND METHODS

Materials and Preliminary Handling

Bambara groundnut was purchased, cleaned and freed of all extraneous materials as well as damaged nuts prior to use. It was washed twice with ordinary water, rinsed with distilled water and cooked at 100^0C ± 5 for 10 minutes to eliminate existing microflora prior to fermentation. The cooked nuts were allowed to cool down to 35^0C ±2 before inoculation with starter cultures. 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.

Reactivation of the Starter Culture

The freeze dried cells of *Lactobacillus plantarum* [NRRL B-4306] and *Lactobacillus fermentum* [NRRL B-1932] was brought to active state by growing in 25 ml Nutrient Broth and incubated in CO_2 enriched jars for 24 hours and centrifuged at 3600-x g for 15 minutes. The recovered cells were rinsed using 10 ml sterile distilled water and centrifuged twice at 3600-x g for 15 minutes. 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 hours of incubation period in CO_2 enriched jars, the colonies on each plate of dilution factor was counted and the plate with approximately 10^3 cfu/ ml was noted and used at every inoculation of the fermentation process.

Bioprocessing of Bambara Groundnut to Flour

Twenty kilogram (20 kg) of cooked Bambara groundnut was submerged in a basin containing 30 liters of sterile distilled water and 10 ml inoculum suspension of *Lactobacillus plantarum* [NRRL B-4306] and *Lactobacillus fermentum* [NRRL B-1932] containing approximately 10^3 cfu/ml was inoculated aseptically. The basin was covered completely and allowed to ferment for three days at room temperature. Thus, the non-inoculated 20 kg of the nuts were used as control.

At the end of the fermentation period, the water was drained and the nuts spread on a tray and dried in a cabinet dryer (Sheldon Lab Oven: VWR 1370) at 60^{o}C for 10 hours. To obtain the whole Bambara groundnut flour; the cooked, inoculated and dried Bambara groundnuts and the cooked, non-inoculated dried sample were milled using commercial attrition grinder (Krupps Model 16409). During milling the nuts were reintroduced into the mill 3 times and the milled flour was 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 utilized for further experiments.

Determination of Amino Acid Profile

The amino acids compositions of the samples was measured on hydrolysates using amino acids analyzer based on high performance liquid chromatography technique according to the method of Dhillon *et al*. (2014).

Hydrolysis of proteins/peptides into amino acids: The vacuum-dried samples were hydrolyzed with 200 µL of constant boiling 6NHCl and 40 µL of phenol through vapour-phase hydrolysis. The samples were dried in an oven at 115 ºC for 20 hours. After completion of hydrolysis, excess HCl was wiped off and the tubes vacuum dried for 90 min. The samples were reconstituted with 100 µL of 20 mM boiling HCl.

Derivatization of amino acids: The reconstituted 20 µL samples were derivatized with AccQ-Fluor reagent kit (WAT052880-Waters Corporation, USA). The AccQ-Fluor borate buffer (60 µL) was added in the sample tube with micropipette and vortexed. Thereafter, 20 µL of AccQ-Fluor reagent was added and immediately vortexed for 30 seconds, and the contents transferred to maximum recovery vials. The vials were heated for 10 minutes in a water-bath at 55 ºC before separation of amino acids using HPLC.

Separation of amino acids: The AccQ-Fluor amino acid derivatives were separated on a Waters 2707 Module HPLC System attached to a PDA (Model PDA 2998). A 10 µL sample was injected into a Waters reversed phase AccQ Tag Silica-bonded Amino Acid Column C18 (3.9 mm x 150 mm) using auto sampler (Waters 2707). The Waters AccQ Tag Eluent A Concentrate (WAT052890) was diluted to 10% in Milli-Q water and used as eluent A, and 60% acetonitrile as eluent B in a separation gradient with a flow rate of 1.0 mL/min. The separation gradient used was 0-2 min (100% A), 2.0 min (98.0% A), 15.0 min (93.0% A), 19.0 min (90.0% A), 32.0 min

(67.0% A), 38.0 min (0.0% A), and 56.0 min (100.0% A). The amino acids were detected using PDA at 254 nm with the column condition set at 37 ºC. The amino acid peaks was acquired using Empower Pro Software® by Waters Corporation (2005-08) and was calculated based on amino acid calibration standard (Thermo Scientific Amino Acid Standard H, Prod # NCI0180) and run at five concentrations 10, 20, 30, 40 and 50 µL having 2.5 µ moles/mL of L-forms of alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine HCl, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine, and 1.25 µ moles/mL of cystine in 0.1NHCl. Injections (10 µL) of 10, 20, 30, 40 and 50 µL of amino acid standard corresponded to 100, 200, 300, 400 and 500 pmol, respectively, of each amino acid, except cystine which had half of their concentrations. The proportional molar concentration for each amino acid was calculated based on the concentration of standard amino acids and expressed as µ g amino acid/mg sample.

Determination of Total and Extractable Minerals

Total minerals were extracted from the samples by the dry ashing method described by Chapman and Pratt (1982). A sample of 2.0 g was acid-digested with diacid mixture (HNO_3:$HClO_4$, 5:1, v/v) in a digestion chamber. The digested samples were then dissolved in double-distilled water and filtered (Whatman No. 42). The filtrate was made to 50 mL with double-distilled water and used for the determination of total calcium, phosphorus and iron.

Calcium was determined by a titration method. Iron was determined by atomic absorption spectrophotometer. Phosphorus and other minerals was determined spectrophotometrically using molybdovanadate method. Extractable minerals in the samples were extracted by the method described by Chauhan and Mahjan (1988). A quantity of 1.0 g of the sample was shaken with 10 ml of 0.03 M HCl for 3 hours at 37^{O}C and then filtered. The clear extract obtained was oven-dried at 100^{0}C and then diacid-digested. The amounts of extractable minerals were determined by the methods described above. While HCl extractability (%) was determined as follows:

$$\text{Mineral extractability (\%)} = \frac{\text{Mineral extractable in 0:03N HCl (mg/100g)}}{\text{Total minerals (mg/100g)}} \times 100$$

Data Analysis

Data generated were analyzed using one-way analysis of variance and mean separation was done by Duncan's new multiple range test and paired t-tests. Significant difference was accepted at $p < 0.05$.

RESULTS

Table 1 shows the amino acid composition of non-inoculated and inoculated Bambara groundnut flour. It was observed that glutamic acid, aspartic acid and leucine are the most abundant amino acids in both samples. Amino acid contents were slightly increased after bioprocess of the Bambara groundnut. The results obtained for the non-inoculated and inoculated samples were compared against the WHO/FAO daily dietary recommendation guideline.

Table 1: Amino acid profile of the flour samples

Amino Acids (mg/100g)	**Non-inoculated**	**Inoculated**	**FAO/WHO Reference***
Alanine	3.90	5.00	-
Arginine	5.00	6.20	2.00
Aspartic Acid	5.62	6.80	4.00
Cystine	0.6	0.92	-
Glutamic Acid	17.20	19.50	-
Glycine	3.35	3.80	-
Histidine	2.50	3.00	2.40
Isoleucine	3.80	4.15	4.20
Leucine	7.00	8.00	4.80
Lysine	2.90	4.50	4.20
Methionine	2.80	3.20	2.20
Phenylalanine	4.80	5.10	2.80
Proline	3.80	4.50	-
Serine	2.60	3.80	-
Threonine	2.60	4.00	2.60
Tyrosine	3.50	3.90	1.40
Valine	4.10	4.85	4.20

Values are mean of triplicates
*Source: FAO (1982)

The bioprocessed Bambara groundnut flour was also found to be rich in calcium. Calcium content of flour from non-inoculated nuts was 220.30 mg/100 g (Table 2) which decreased to 198.80 mg/100 g after fermentation. The results indicated that cooking and fermentation of the nut, significantly ($p < 0.05$) reduced the calcium content of Bambara groundnut. The loss of calcium during the treatment may be attributed to its leaching out into the discarded water used for cooking and fermentation. HCl-extractability of calcium in non-inoculated sample was found to be 80.50%. The treatment significantly increased HCl-extractability of iron ($p < 0.05$) from the control value of 65.28% to 75.80%.

Table 2: Total and extractable minerals of the flour samples

Minerals	Non-inoculated		Inoculated	
	Total (mg/100 g)	Extractable (%)	Total (mg/100 g)	Extractable (%)
Cu	$0.30^{b}\pm0.10$	$65.80^{a}\pm0.30$	$0.20^{b}\pm0.20$	$68.20^{a}\pm0.90$
Fe	$5.90^{c}\pm0.30$	$65.30^{b}\pm0.70$	$3.80^{d}\pm0.20$	$75.80^{a}\pm0.10$
Mn	$3.00^{c}\pm0.60$	$75.10^{b}\pm0.50$	$1.90^{d}\pm0.40$	$84.20^{a}\pm0.30$
Zn	$7.70^{c}\pm0.30$	$58.30^{b}\pm0.10$	$3.80^{d}\pm0.20$	$70.10^{a}\pm0.80$
P	$265.80^{a}\pm0.40$	$65.10^{d}\pm0.20$	$248.40^{b}\pm0.60$	$70.10^{b}\pm0.10$
Ca	$220.30^{a}\pm0.80$	$80.50^{d}\pm0.60$	$198.80^{b}\pm0.20$	$89.90^{c}\pm0.50$
K	$50.20^{c}\pm0.90$	$65.40^{b}\pm0.30$	$38.90^{d}\pm0.20$	$80.50^{a}\pm0.20$
Na	$11.50^{c}\pm0.50$	$72.30^{b}\pm0.10$	$7.80^{d}\pm1.00$	$85.80^{a}\pm0.80$

Values are means ± SD (n = 3). Values on the same row with different superscripts are significantly ($p < 0.05$) different

DISCUSSION

The amino acid profile of the starter culture inoculated sample was found to slightly increase after fermentation with *Lactobacillus plantarum* [NRRL B-4306] and *Lactobacillus fermentum* [NRRL B-1932]. Similar observation has been reported by Olaofe and Akintayo (2000) and Adeyeye and Afolabi (2004) for soaking and cooking of Bambara groundnut; glutamic acid was the most concentrated essential amino acid (17.00%).

Other researchers using various microorganisms for fermentation other than the ones used in this study have also reported similar observations on the increasing effects of fermentation on amino acids content. According to Sarkar *et al.* (1997), *Bacillus* fermented soybean led to an increase in

free amino acids and ammonia by 60- and 40-fold, respectively. Baumann and Bisping (1995) found that certain *Rhizopus* strains with high proteolytic activity were able to release nearly 5 times more amino acids. Ferial and Esmat (2011) observed 21.8% increases in essential amino acids in fermented chickpea with *Rhizopus* at 48 hours of fermentation. Song *et al.* (2008) reported that fermentation of soybean with *L. plantarum* and *B. lactis* caused increase in amino acids content while fermentation with *S. cerevisae* showed the opposite results. When comparing the essential amino acids in Bambara groundnut flour with the recommended FAO/WHO provisional pattern, the nut was superior with respect to aspartic acid, threonine, methionine, leucine, tyrosine, phenylalanine, hstidine and arginine and adequate in valine and isoleucine.

The fermentation of Bambara groundnut with *Lactobacillus plantarum* [NRRL B-4306] and *Lactobacillus fermentum* [NRRL B-1932] was also found to reduce the total mineral content and increase the mineral extractability of the sample. The results are in close consistence with the results of Duhan *et al.* (2002) who also reported a significant decline in the total calcium content on water soaking. All other major minerals followed a trend similar to that obtained for calcium. The iron content of non-inoculated sample was 5.90 mg/100 g; bioprocessing of the seeds reduced iron content to 3.80 mg/100 g. The reduction in iron content may be due to loss of iron in the fermentation medium. The results are in agreement with those of Lestienne *et al.* (2005), who observed reduction in iron content of the soaked grains as compared to raw ones.

HCl-extractability of calcium in non-inoculated sample was found to be 80.50%. Extractable calcium level, after bioprocess significantly increased to 89.90%. Divalent cations, such as Ca, are generally present in association with phytic acid; this may be responsible for its lower extractability. However, reduction in phytic acid as a result of fermentation may explain higher HCl-extractability of calcium and other minerals as observed by Duhan *et al*. (2002). HCl-extractability of all other major minerals followed a trend similar to that obtained for calcium. The treatment significantly increased HCl-extractability of iron ($p < 0.05$) from the control value of 65.28% to 75.80%. HCl-extractability of all other trace minerals followed a trend similar to that obtained for iron. As a divalent cation, Fe is also generally present in association with phytic acid and this may be responsible for its lower extractability. However, reduction in phytic acid and other antinutrients as a result of bioprocess may explain higher HCl-extractability of iron and other trace minerals as observed by Duhan *et al*. (2002).

CONCLUSION

With the recent increasing global economic recession and need for effective food security measures, the production and use of this often neglected crop should be improved to reduce dependence on imported flours. Owing to the outstanding potentials of Bambara groundnut in helping to curb food security issues, this study has produced a bioprocessed Bambara groundnut flour and when compared to the non-inoculated flour, the bioprocessed flour showed improved amino acid profile when compared to the recommended FAO/WHO provisional pattern and increased mineral extractability when compared to the total mineral content.

Generally, a significant increase in the soluble fraction of a food is observed during fermentation. The quantity as well as quality of the food proteins as expressed by biological value is generally increased, often due to the reduction in anti-nutritional factors. The study suggests that microbial fermentation may offer a simple means to improve the nutritional pattern of this crop, hence, optimize its utilization.

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