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Research Article
Change in Microbial Ecology of Bambara Flour by Lactic Acid Bacteria Consortium During Fermentation and its Effect on Anti-nutritional Factors

[1]Alloysius Chibuike Ogodo, [2]Ositadinma Chinyere Ugbogu, [2]Reginald Azu Onyeagba and [2]Hope Chukwuemeka Okereke

[1]Department of Microbiology, Faculty of Pure and Applied Sciences, Federal University Wukari, P.M.B. 1020 Wukari, Taraba State, Nigeria
[2]Department of Microbiology, Faculty of Biological and Physical Sciences, Abia State University, P.M.B. 2000 Uturu, Abia State, Nigeria

Abstract

Background and Objective: Anti-nutritional factors such as phytate, tannin, polyphenols and trypsin inhibitors bind to nutrients and prevent their bioavailability. This study investigated the effect of alteration in the natural ecology of microorganisms by lactic acid bacteria (LAB)-consortium on the anti-nutritional factors of bambara groundnut flour during fermentation. **Materials and Methods:** Bambara groundnuts were processed into flour, fermented spontaneously and with LAB-consortium (*Lactobacillus rhamnosus*, *Lactobacillus reuteri*, *Lactobacillus plantarum*, *Lactobacillus nantensis*, *Lactobacillus fermentum*, *Pediococcus acidilactici* and *Lactobacillus brevis*) previously isolated from maize and sorghum. **Results:** The result showed significant ($p<0.05$) decrease in tannin, phytate, polyphenol and trypsin inhibitor activity with increasing fermentation period. The decreases in the anti-nutritional factors were more in the samples fermented with LAB consortium from maize and LAB consortium from sorghum than spontaneously fermented sample. **Conclusion:** This suggested that altering microbial ecology of bambara flour by LAB-consortia during fermentation has potential in decreasing the anti-nutritional factors and improve bioavailability of nutrients.

Key words: Anti-nutritional factors, bambara flour, microbial ecology, fermentation, LAB-consortium

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Corresponding Author: Alloysius Chibuike Ogodo, Department of Microbiology, Faculty of Pure and Applied Sciences, Federal University Wukari, P.M.B. 1020, Wukari, Taraba State, Nigeria Tel: +234806666383

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Food can be preserved, important nutrients produced and anti-nutrients eliminated by fermentation. Moreover, during fermentation, chemical compounds are changed and foods are made more edible by predigesting the foods[1]. Plants that contain poisonous substances like cassava are converted to edible products by fermentation. Tubers, cereals and legumes used for the production of various fermented food may contain significant amount of toxic substances and anti-nutritional factors such as phytic acid, tannins, cyanogenic glycosides, lectins, oxalic acid, trypsin inhibitors, chymotrypsin inhibitors and alpha-amylase inhibitors. These substances interfere with mineral bioavailability, digestibility of proteins and carbohydrates, thereby reducing the nutrient value of the food. However, natural and mixed cultures of bacteria and fungi are capable of reducing the anti-nutritional factors in food during fermentation[2,3].

Fermentation increases nutritional value of food. For instance, porridge made from grains after fermentation have increased nutritional value to the extent of reducing the risk of diseases in children; probiotic supplements are capable of fighting cancer and other diseases, fermented vegetables and milk have more vitamin C and B, respectively[4]. The health benefits of fermented foods are mostly through the lactic acid fermentation. Report has shown that *Lactobacillus rhamnosus* and *Lactobacillus reuteri* could colonize the vagina and reduce the risk of bacterial vaginosis[5]. The health and therapeutic effect of the lactic acid bacteria are attributed to changes in gastrointestinal microflora to suppress the growth of pathogens[3,5,6].

One of the major benefits of lactic acid bacteria (LAB) fermentation is detoxification. Lactic acid bacteria by fermentation reduces anti-nutritional factors such as phytates, tannins, polyphenols, trypsin inhibitors etc. and detoxifies toxins in more advantageous way than other processes like cooking and roasting because it preserves the nutritive value and flavor of food[7]. Moreover, fermentation irreversibly degrades mycotoxins without leaving any toxic residue and without adversely affecting the nutritional value of the food[1]. The LAB also confers preservative effects on food through lowering of pH, acid production and other antimicrobial substances in the food[8].

Bambara groundnut as indigenous crop to Africa is one of the neglected and under-utilized crop. However, it plays important role in the diet of many people of Africa especially Nigeria where it is utilized as a source of calories, proteins and minerals[9]. The seed contains sufficient amount of carbohydrate, protein (higher in methionine) and oil (made of unsaturated fatty acid, palmitoleic, oleic and caprylic acid) and compares with cowpea, chicken pea and faba beans in nutritional attributes[10-13]. However, Bambara groundnut as a legume is rich in anti-nutritional factors associated with fibres which bind to nutrients and minerals reducing their bioavailability. This serves as a major hindrance to their effective utilization[14,15]. Hence, the present study evaluated the effect of lactic acid bacteria consortium fermentation on the anti-nutritional factors of Bambara groundnut flour. Moreover, there are no literature that has evaluated the effect of lactic acid bacteria consortium fermentation on bambara groundnut flour which holds potential for bambara flour with practical applications in food industries for commercialization.

MATERIALS AND METHODS

Collection of sample and preparation: Bambara groundnut (*Voandzeia subterranean* L.) was obtained from Mushin market, Lagos State, Nigeria and transported to the laboratory in a clean polythene bag for identification and analysis at Federal Institute of Industrial Research Oshodi (FIIRO). Lactic acid bacteria were previously isolated from fermenting maize and sorghum. The raw cream-coloured bambara groundnut was freed of foreign materials, washed and rinsed with distilled water. The sample was dried with hot air oven (GL, England) at 60°C for 8 h and milled into powder using laboratory disc mill and then stored in clean air tight containers at 4°C for further use[16].

Choice of inoculum and preparation of starter culture: The inoculum was selected based on their reported benefits[17-19], tolerance to acid, salt, lowering of pH, level of acid production and growth on nutrient depleted medium after a pre-fermentation study. The starter culture was prepared according to the method described by Ogodo *et al.*[20] with slight modification. Five lactic acid bacteria previously isolated from each of fermenting maize and fermenting sorghum were combined as follows, *Lactobacillus plantarum* WCFS1+*Lactobacillus rhamnosus* GG, ATCC 53/03+ *Lactobacillus nantensis* LP33+*Lactobacillus fermentum* CIP 102980+*Lactobacillus reuteri* DSM 20016, (consortium from maize) and *Pediococcus acidilactici* DSM 20284+*Lactobacillus fermentum* CIP 102980+*Lactobacillus brevis* ATCC 14869+ *Lactobacillus nantensis* LP33+*Lactobacillus plantarum* WCFS1, (consortium from sorghum). The combined LAB were, respectively grown in an Erlenmeyer flasks containing 210 mL de-Man Rogosa and Sharpe broth and incubated for 48 h in an orbital shaker incubator (REMI/396LAG) at 37°C for the inoculum to build-up. The inocula were harvested and washed

by centrifugation at 5000 rpm for 10 min and then maintained in fresh MRS broth before fermentation. The washed cells were diluted to obtain 0.5 McFarland standard using sterile distilled water.

Fermentation of bambara flour: Fermentation of the Bambara groundnut flour was carried out following a slight modification of the method of Ogodo *et al.*[21]. About 500 g of the uncooked flour was mixed with distilled water in the ratio of 1:2 w/v in sterile fermentation container. Inhibition of fungi and other contaminating micro-organisms (verified by no observable growth on plate count agar after 18-24 h of incubation) was done by addition of 0.5 g L^{-1} of potassium sorbate to the mixture. The mixture was inoculated with 10 mL of 1.0×10^8 cells mL^{-1} of the mixture of the lactic acid bacteria suspension and allowed to ferment. The control trial was prepared following the same method but potassium sorbate and the starter organisms were not added for spontaneous fermentation. Samples were withdrawn at 0, 12, 24, 36 and 48 h for analysis.

Determination of anti-nutritional factors: Tannins were determined according to the method described by Onyango *et al.*[22] with slight modification. Exactly 10 mL of 4% HCl in methanol was added to 0.25 g of ground sample in Erlenmeyer flasks and closed with paraffin. The flasks were shaken gently for 20 min in a shaker and the resulting extracts centrifuged for 10 min at 4500 rpm. About 5 mL of 1% HCl in methanol was added to the residue from the first extraction to obtain the second extraction. The first and second extracts were combined and made up to 25 mL. Catechin standard solutions were prepared using methanol from 100-1000 ppm. Exactly 1 mL of each extract and 1 mL of each respective standard were pipetted to a corresponding labeled test tube and exactly 5 mL of vanillin-HCl reagent added. The blank was prepared by adding 5 mL of 4% HCl in methanol to 1 mL of the aliquot extracts in test tubes. The absorbencies of the standard solutions, sample extracts and blanks were read in a UV754 spectrophotometer (HospiBrand, USA) at 500 nm. The percentage catechin equivalents (CE%) were calculated as follows:

$$\text{CE } (\%) = \frac{\text{CC} \times \text{VM}}{\text{VE} \times \text{Wt}} \times 100$$

Where:
CC = Catechin concentration
VM = Volume
VE = Volume of extract
Wt = Weight of sample

Liquid chromatography method of phytic acid determination as described by Onyango *et al.*[22] was used to determine the phytate composition of the flour. Total phenolic content of the sample was determined with Folin-ciocalteu reagent according to the method described by Akond *et al.*[23] with slight modification. The flour sample was extracted using 5 mL of 50% methanol/water and 5 mL of 1.2 M HCl in 50% methanol/water with heating at 90°C for 3 h, cooled and then diluted to 10 mL with methanol. This was centrifuged for 5 min at 5000 rpm. Exactly 1 mL of Folin-ciocalteu reagent was added to 50 µL of the extract solution in a test tube and mixed thoroughly. About 1 mL of 10 % Na_2CO_3 was added to the mixture after 3 min and then allowed to stand for 1 h in the dark. Absorbance was measured at 760 nm in UV754 spectrophotometer (HospiBrand, USA). The concentration of total phenolic compounds in the flour extract was determined as micrograms of gallic acid equivalent and expressed as mg/100 g gallic acid equivalent of dry mass.

Trypsin inhibitor activity was determined by the method described by Mbata *et al.*[24] with slight modification. Exactly 1 g of the sample was extracted by soaking overnight at 4°C in 50 mL 0.01 NaOH with pH adjusted to 8.4. Synthetic benzoyl DL arginine-p-nitroanilide (BAPNA) was used as substrate. Residual enzyme activity was determined in systems containing 2 mL aliquots of the sample extracts by measuring the absorbance at 410 nm in UV754 spectrophotometer (HospiBrand, USA). Trypsin inhibitor activity (TIA) in term of milligrams pure trypsin sample was calculated as:

$$\text{TIA} = \frac{2.632 \times \text{D} \times \Delta 1}{\text{S}}$$

Where:
1 = Change in absorbance due to trypsin inhibition/mL diluted sample extract
D = Dilution factor
S = Weight of sample (g)

Statistical analysis: All the analyses were conducted in triplicate and the mean data±SD (standard deviation) were reported. Data were subjected to analysis of variance (ANOVA) for repeated measurements. Each of the parameters analyzed were compared between spontaneous fermentation, LAB consortium from maize and LAB consortium from sorghum fermentations respectively using statistical package for the social sciences (SPSS) version 20.0 software. Significance was accepted at $p \leq 0.05$.

RESULTS

The result of the effect of fermentation on the tannins (Catechin Equivalent, CE%) of the Bambara groundnut flour was presented in Fig. 1. It was observed that the tannins composition of the sample decreased significantly ($p<0.05$) with increasing fermentation periods. The decrease ranged from 4.63±0.061% (0 h) to 0.08±0.00% (48 h LAB-consortium from sorghum fermented sample). The highest decrease was observed in LAB-consortium from sorghum fermented sample, followed by LAB-consortium from maize fermented sample while the spontaneous fermentation was the least.

As Fig. 2 presented the effect of change in microbial ecology fermentation by lactic acid bacteria consortium on the phytic acid composition of Bambara groundnut flour. The result indicated that the phytate composition decreased significantly ($p<0.05$) with increasing fermentation time. The decrease ranged from 46.01±0.04 to 16.22±0.12 mg/100 g (spontaneous fermentation), 46.01±0.04 to 12.86±0.06 mg/100 g (LAB-consortium from maize fermented sample) and LAB-consortium from maize fermented sample from 46.01±0.04 to 13.04±0.06 mg/100 g (48 h LAB-consortium from sorghum fermented sample). The variations differ significantly ($p<0.05$) when compared between natural, LAB-consortium from maize and LAB-consortium from sorghum fermented samples respectively. On the whole, the lowest reduction in phytate was observed in LAB-consortium from maize fermented samples at 48 h for all the substrates.

The effect of change in the microbial ecology of Bambara flour during fermentation by LAB-consortium on polyphenol composition shows a significant ($p<0.05$) decrease with increasing fermentation time (Fig. 3). The decrease ranged from 684.86±0.03 to 223.62±0.60 mg/100 g, 684.86±0.03 to 167.46±0.06 mg/100 g and 684.86±0.03 to 171.54±0.18 mg/100 g in spontaneous, LAB-consortium from maize and LAB-consortium from sorghum fermentations respectively. The lowest value was obtained in the LAB-consortium from maize fermented sample (167.46±0.06 mg/100 g). The values obtained at the various time interval differ significantly when compared between the spontaneous fermentation and the LAB-consortia fermentations at all-time intervals.

The effect of change in the microbial ecology of Bambara flour by LAB-consortium during fermentation on the trypsin inhibitor activity shows a significant decrease ($p<0.05$) with

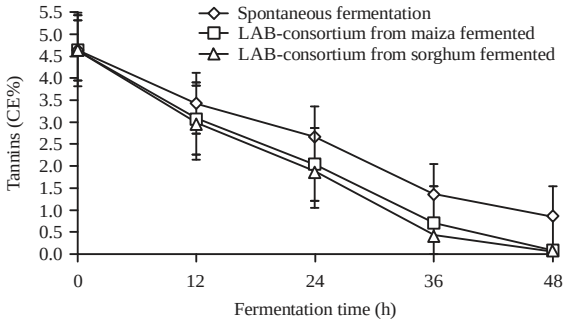

Fig. 1: Effect of change in microbial ecology of Bambara flour by LAB-consortium during fermentation on the tannin content. Each point represents the mean of three independent experiments and error bars indicate±SE. There were significant differences ($p<0.05$) at each successive time interval when natural fermentation is compared to LAB consortia fermentations

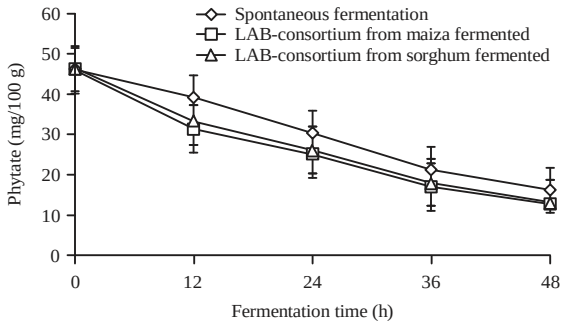

Fig. 2: Effect of change in microbial ecology of Bambara flour by LAB-consortium during fermentation on the phytate content. Each point represents the mean of three independent experiments and error bars indicate±SE. There were significant differences ($p<0.05$) at each successive time interval when natural fermentation is compared to LAB consortia fermentations

increasing fermentation periods. The values were observed to decrease from 7.40±0.06 to 1.46±0.04 mg/100 g (spontaneous fermentation), 7.40±0.06-0.99±0.03 mg/100 g (LAB-consortium from maize fermentation) and from 7.40±0.06 to 1.01±0.06 mg/100 g (LAB-consortium from sorghum fermentation) (Fig. 4). The decreases differ significantly ($p<0.05$) when compared between naturally fermented, LAB-consortium from maize and LAB-consortium from sorghum fermented samples. The lowest value of polyphenol was observed in LAB-consortium from maize fermented samples at 48 h.

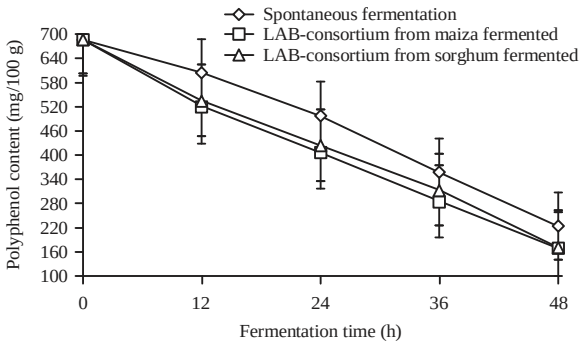

Fig. 3: Effect of change in microbial ecology of Bambara flour by LAB-consortium during fermentation on the polyphenol content. Each point represents the mean of three independent experiments and error bars indicate±SE. There were significant differences ($p<0.05$) at each successive time interval when natural fermentation is compared to LAB consortia fermentations

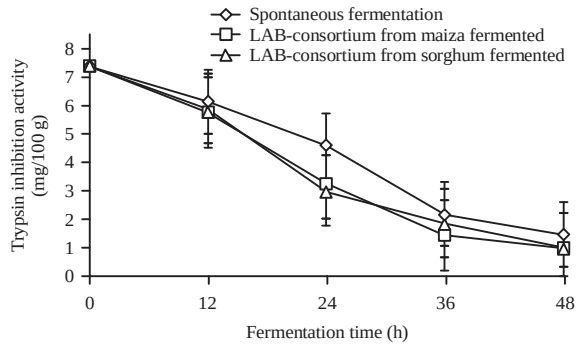

Fig. 4: Effect of change in microbial ecology of Bambara flour by LAB-consortium during fermentation on the trypsin inhibitor activity. Each point represents the mean of three independent experiments and error bars indicate±SE. There were significant differences ($p<0.05$) at each successive time interval when natural fermentation is compared to LAB consortia fermentations

DISCUSSION

The effect of change in the microbial ecology of Bambara groundnut flour by LAB-consortium during fermentation on the tannin (%) content shows a significant ($p<0.05$) decrease with increasing fermentation periods. The decrease ranged from 4.63±0.061% (unfermented) to 0.08±0.00%, respectively. On the whole, the lowest reduction in tannins was observed in LAB-consortium from sorghum fermented samples at 48 h. According to Roger *et al.*[25], some LAB including *Lactobacillus plantarum*, *Lactobacillus paraplantarum* and *Lactobacillus pentosus* are capable of degrading tannins through their acetylhydrolase tannin activity which also confers ecological advantage to the LAB. Onwurafor *et al.*[26], reported reduction in tannin content of mungbean as a result of spontaneous fermentation from 1.13-0.81 mg/100 g. According to Adeyemo and Onilude[27], fermentation with *Lactobacillus plantarum* reduced the tannin content of soybean from 1.93-0.12 mg g^{-1}. Olanipekun *et al.*[15], reported decrease in tannins content of bambara groundnut as a result of fermentation which also agreed with the work of Afolabi *et al.*[28] on papaya seed. The reduction in the present study for bambara groundnut in consortia fermentations are lower than the 0.16 mg/100 g reported by Abiodun andAdepeju[29]. The decrease in tannin as a result of fermentation could be attributed to microbial phenyl oxidase action or activity of enzymes associated with the seeds[26,30]. Also, it could be as a result of the degradation of the anti-nutrients into smaller units by the action of enzymes mobilized during fermentation as well as the activity of alpha-galactosidases as reported by Adeyemo and Onilude[27]. Tannins have stringent and bitter taste which affects palatability and form complex with protein thereby reducing their digestibility[31]. Therefore, reduction in its content by fermentation would improve its nutritional value. Moreover, the present study shows lowest values of tannins in set-ups with change in their natural microbial flora by LAB-consortium, an indication of the effectiveness of LAB in reducing tannin content of Bambara.

Phytic acids are commonly found in seeds and grains, cereals, legumes and other plants where they constitute the main storage form of total phosphorus. Because of the high negative charges, phytic acids can form complex with protein and minerals and lead to a decrease in protein solubility and minerals availability[31]. In the present study, fermentation caused a significant ($p<0.05$) decrease in the phytate composition of the various samples with increasing fermentation time. The decreases were observed more in fermentations set-ups whose natural microbial flora was changed with LAB-consortium (LAB-consortium from maize and from sorghum respectively). The reduction in the phytate content observed in the present study could be due to the fact that LAB produces phytases and phosphophytases which help to hydrolyse phytates to inositol and orthophosphates. Moreover, the low pH observed during LAB fermentation favours phytase activity[25,32]. Cereals and legumes are major sources of phytate in diets which are undegraded during transit through the gastrointestinal tract and form insoluble complexes which do not provide absorbable essential elements. Therefore, degree of phytate degradation can affect

the nutritional value of a phytate-rich diet[33,34]. Phytates are known to inhibit iron-driven hydroxyl radical formation due to formation of catalytically inactive iron chelate[34]. In its natural form as phytate-mineral-protein complex, phytate decreases the availability of zinc, manganese, copper, molybdenum, calcium, magnesium and iron, as well as affects digestibility of vitamins and minerals[35] Osman[30], reported that 24 h fermentation caused a significant ($p<0.05$) decrease in the phytic acid content of pearl millet from 647.0-310.95 mg/100 g while Onwurafor *et al.*[26] reported decrease in phytate content of mung beans after fermentation. The finding of the present investigation is similar to the work of Babalola and Giwa[35], who reported decrease in phytate content of soybean after fermentation although a lower value of 17.8 mg/100 g was recorded before fermentation. Phytic acid content of Bambara groundnut has been reported to decrease during fermentation with *Rizopus* species[15].

In the present study, the polyphenol content of bambara groundnut flour decreased significantly ($p<0.05$) with increasing fermentation time. The decreases were observed to be more in fermentations by the LAB-consortia with LAB- consortium from maize fermented samples having the lowest value at 48 h. This is an indication that changing the natural microbial flora of the flour with LAB-consortium can effectively reduce their polyphenol content. Similar observations have been reported in maize[25], millet[36] and sorghum lines[32]. The reduction in polyphenols content after fermentation could be due to the action of tannase enzyme and polyphenol oxidase released by the fermenting micro-organisms[25,34].

The trypsin inhibitor activity (TIA) of the flour in the present study decreased after spontaneous, LAB-consortium from maize and LAB-consortium from sorghum fermentations decreased significantly ($p<0.05$) with increasing fermentation periods. The decrease ranged from 7.40 ± 0.06 to 0.99 ± 0.03 mg/100 g. The decreases differ significantly ($p<0.05$) when compared between natural fermentation and LAB-consortia fermentation. The lowest reduction in TIA was observed in LAB-consortium from maize fermented samples at 48 h. This finding is similar to the report of Osman[30] and Osman and Gassem[31] on pearl millet and sorghum, respectively. Adeyemo and Onilude[27] showed that fermentation with *L. plantarum* isolated from fermenting cereals decreased the trypsin inhibitor activity of soybean from 1.20-0.01 mg g^{-1} after five days. Ari *et al.*[1], also reported decrease in trypsin inhibitor activity (TIA) with fermentation in soybean. This finding also agreed with the work of Olanipekun *et al.*[15], who reported that fermentation cause reduction in TIA of bambara groundnut fermented with species of *Rhizopus* and consistent with previous report[37]. However, the present study did not conform to the report of Fadahunsi[38], who reported increase in TIA after 15 h fermentation of Bambara nuts. Osman[30], reported that trypsin inhibitor activity is implicated as one the factors responsible for reducing protein digestibility, pancreatic hypertrophy and poor growth performance in rat, mice and chicks. Hence reduction in trypsin inhibitor activity (TIA) could be useful in improving nutritional quality of Bambara flour.

CONCLUSION

The present study showed that anti-nutritional factors, tannin, phytate, polyphenol and trypsin inhibitor activity decreased significantly ($p<0.05$) during spontaneous and LAB-consortia fermentations. The highest decreases were observed in the samples fermented with the LAB-consortia in all parameters analyzed. The LAB-consortium from maize and LAB-consortium from sorghum fermented samples did not differ significantly ($p>0.05$) in all parameters analyzed. This suggested that change in microbial ecology by LAB-consortium during fermentation is an effective means of reducing the anti-nutritional factors thereby improving the nutritional bioavailability and digestibility more than natural or spontaneous fermentation. However, further work can be done in optimization and scale-up of the process for commercialization of the product.

SIGNIFICANCE OF THE STUDY

Legumes such as bambara groundnut are rich in anti-nutritional factors which bind to nutrients and minerals thereby reducing their bioavailability. This serves as a major hindrance to their effective utilization. Therefore, reduction in the anti-nutritional factors will enhance the bioavailability of the nutrients and minerals. Lactic acid bacteria consortia were used to reduce the anti-nutritional factors in bambara groundnut flour. The research has the potential to be applied in food industries to improve the quality of legumes flour. Also, it has potential for product commercialization. Moreover, there are no literature that has evaluated the effect of lactic acid bacteria consortium fermentation on bambara groundnut flour

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