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Functional and Rheological Profile of LAB-fermented Bambara Groundnut (*Vigna subterranean* (L)) Flour

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Lactic fermentation is commonly employed to improve protein digestibility and overall nutritional quality of grains foods. This study evaluated the functional and rheological properties of flour samples from Bambara groundnut fermented with *Lactobacillus plantarum* [NRRL B-4306] and *Lactobacillus fermentum* [NRRL B-1932] obtained from the United States Department of Agriculture. Functional profile such as particle size index, water absorption capacity, swelling capacity, and least gelation concentration of the flour were determined; as well, amylograph and maturograph evaluations were used to determine the rheological properties and the results presented as average, minimum, and maximum values. Particle size determination observed that 150, 125, 105 µ orifice did not readily accommodate particles from the non-inoculated samples while the inoculated samples passed through 150 and 125 µ but did not readily pass through 105 µ orifice. Bioprocess with lactic acid bacteria increased the water absorption capacity of the flour samples from 346.5 to 386.4%, the least gelation concentration decreased from 5.3 to 4.1%, while swelling capacity increased from 14.9 to 23.2 mg/100 g for non-inoculated and inoculated flours,

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respectively. Rheological investigations show evaluations for amylograph and maturogram determinations. Values obtained for amylograph indicate that temperature at start of gelatinization was peak at 63.8 and 63.0^{0}C for non-fermented and LAB-fermented flour with no significant (p < 0.05) difference, while temperature at maximum viscosity and maximum viscosity had significant (p < 0.05) values of 92.1 and 76.0^{0}C, as well as 730 and 265 brabender units (BU), respectively. Thus, amylograph quality of the fermented flour sample was indicated by the maximum viscosity which is significantly higher in the case of non-inoculated flour sample. The maturograph evaluation also recorded the maturation behavior of the dough prepared from the test flours after the proofing time (fermentation rest) by means of a sensing probe which records the elasticity of the mature dough every 2 min and produces the typical zigzag form of the maturogram. This action was recorded in maturograph units (MU) on the strip-chart with values of 44 and 28 min for final proof time, 750 and 610 MU for dough level, 210 and 220 MU for dough elasticity, as well as 10 and 12 min for proofing stability, determined respectively for non-fermented and LAB-fermented flour samples. LAB-fermentation demonstrated to improve the functionality and rheology of Bambara groundnut flour and the production process could be further controlled to achieve products of optimal quality.

Keywords: Fermentation; functional properties; rheology; Bambara groundnut; flour.

1. INTRODUCTION

Bambara groundnut (*Vigna subterranean* (L)) is a grain legume which is grown essentially for human consumption and often eaten as snack or used as an ingredient in cooking [1]. Alternatively, the nuts are ground into flour and blend into many customary dishes. The crop still offers a great contribution to the diet of many parts of Africa [2]. A recent study [3] noted that the nutritional composition of Bambara grains varies with cultivar and growing locations but is considered a complete food because of its high protein content (9.60–40.0%); and is also considered to have a good balance of the essential amino acids [4].

Despite the recent utilization of Bambara groundnut, many researchers still hold the opinion that this legume crop is underutilized irrespective of its numerous nutritional potentials [5,6]. However, some authors believe that this assertion is no longer valid considering the recent advancement in the use of the grain in various food applications [3]. Although Bambara groundnut seems to have attained a better utilization status as evidenced in the literature where the crop has been used to enrich a variety of foods such as infant formula and noodles as demonstrated in our previous works [7,8], there is still need to find adequate techniques in processing to optimize the utilization of this crop and improve its hard-to-cook phenomenon which has limited its production. Researchers have also opinioned that farmers attributed this decline in Bambara groundnut production to lack of adequate processing techniques to enhance its utilization [9]. However, various bioprocess techniques has been utilized to improve food processing and preparation. Among numerous methods; malting, germination, and fermentation are the major efforts made to improve food processing and reduce the amounts of anti-nutrients in various foods [10,9,11, and 12], thus, the most effective treatment is fermentation [13].

Grain legumes and other food crops are fermented for numerous reasons which include but not limited to modification of the nutritional, functional, physical, and sensory value of the food [14,15] with the aim to yield improved quality of the products. Plant foods also contain anti-nutritional substances such as trypsin inhibitors which are known to interfere with protein digestibility, as well as low-molecular weight carbohydrate fractions which induce flatulence when ingested. These anti-nutritional factors are degraded or reduced to safe levels through the process of fermentation [14]. More, food fermentation involves the activities of some enzymes, particularly lipolytic and proteolytic, which enhance the digestibility of raw plant materials [16]. Fermented foods also exhibit characteristic flavor and aroma properties which are considered appealing to consumers. Fermentation using lactic acid bacteria (LAB) has been used to improve the palatability of food products and the availability of proteins and vitamins [7,8,17] and has shown to confer preservative and detoxifying effects on foods [18]. LAB fermentation can also provide an inexpensive processing method that could improve the digestibility of protein in grains and has the potential to increase the protein content

for various foods or feed applications [19]. Hence, the present study aims to evaluate the effect of LAB fermentation on the functional and rheological attributes of Bambara groundnut flour for use in food product development.

2. MATERIALS AND METHODS

2.1 Pre-handling of Bambara Groundnut and Starter Culture

Bambara groundnut and processed wheat flour were purchased from a local market in Enugu, Enugu State, Nigeria. The Bambara groundnut was sorted to remove all damaged nuts and extraneous materials prior to use. As a pretreatment measure, clean water was used to wash the grains and was cooked for 15 min at temperature of 100 ^{0}C to eliminate existing microbial population prior to starter culture inoculation with starter culture. The starter culture used for this study, *Lactobacillus plantarum* [NRRL B-4306] and *Lactobacillus fermentum* [NRRL B-1932] 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 starter culture was obtained in freeze dried state and was brought to active state by inoculating a suspension in 25 mL Nutrient Broth, incubated in CO_2 enriched jars form 18 to 24 hours, and centrifuged for 15 min at 3600-x g to recover cells. The recovered cells were serially diluted and plated using pour plate technique on Plate Count Agar and plates showing growth of approximately 10^6 cfu/mL were used throughout the fermentation process.

2.2 Production of Fermented Bambara Groundnut Flour

Fermented and control Bambara groundnut flour was produced as outlined in our previous studies [7,8,14]. Briefly, 20 kg each of the Bambara groundnuts samples were submerged in 30 liters of sterile distilled water, inoculated with bacterial strains of *Lactobacillus plantarum* [NRRL B-4306] and *Lactobacillus fermentum* [NRRL B-1932] and allowed to stand for 3 days after which the water was drained and the fermented nuts spread on a tray and dried in a cabinet dryer (Sheldon Lab Oven: VWR 1370) at 60^{o}C for 10 hours. Bambara groundnut flour was obtained by milling using commercial attrition grinder (Krupps Model 16409). During milling the nuts were reintroduced into the mill 3 times and the milled flour was stored in an airtight nylon bags and utilized for experiments within 1 month of production. Non-inoculated Bambara groundnut flour was produced using spontaneous fermentation and used as control.

2.3 Determination of Functional Properties

Particle size index: Flour granulation characteristics of the LAB inoculated and non-inoculated Bambara groundnut were determined by particle size analysis using a simple sieve method. As described [20], 100 g each of the flour samples was sieved through orifice of 210, 180, 150, 125 and 105µ. Sieving was carried out continuously for 10 minutes for each process after which the recovered flour from each sieve were carefully collected and weighed.

Water absorption capacity: Evaluation of water absorption capacity (WAC) was done according to the modified method specified by [21] where 32 mL of distilled water were added to 5 g of the flour sample in a beaker and the suspension stirred using magnetic stirrer for 5 minutes. The suspension was transferred into centrifuge tubes and allowed to stand for 10 minutes then centrifuged at 3500 rpm for 30 minutes. The supernatant was decanted and the tubes dried in hot air oven for 15 minutes at 50°C. The dried tubes were weighed and the difference in the initial and dry weight was used to calculate the WAC as a percentage.

Least gelation concentration: Least gelation concentration of the flour samples was determined using the method described by [22]. Suspensions of the flour samples was prepared for 5, 10, 15, 20, 25 and 30% (w/w) in distilled water and 10 mL of each of the prepared dispersions transferred into test tubes. The tubes were heated in a boiling water bath for 1 hour, cooled in a cold water bath and further allowed to cool for 2 hours at 4°C. Least gelation concentration was determined when the sample did not slip or fall from the test tube when it is inverted.

Swelling capacity: The swelling capacity was determined using 20 g of the flour sample weighed into a clean, dry graduated cylinder. The cylinder containing the samples was tapped on a table 2 times and then filled with 80 mL of distilled water. The final volume of the sample was noted after the cylinder was allowed to stand

for 1 hour. The ratio of the final volume to initial volume was used to determine the swelling capacity on volume basis. Thus, the supernatant was decanted and the weight of flour sample and the cylinder observed to determine the swelling capacity on weight basis using the ratio of final weight to initial weight of sample [23].

2.4 Determination of Rheological Properties

The rheological parameters were determined to observe the pasting properties from slurries of the flour samples. The non-inoculated and LAB-fermented Bambara groundnut were evaluated by means of amylograph (Brabender GmbH & Co. KG), according ICC 126 standard; and maturograph (Brabender GmbH & Co. KG), according to the producer recommendations. The rheological investigations were presented by average, minimum, and maximum values.

2.5 Amylograph Evaluation

The ICC 126 standard observed in this study specifies a method, using the Brabender Amylograph, for determination of the gelatinization properties of starch, indicated by the viscosity, of a flour-water suspension during heating. The method is applicable to wheat and rye flours but was used to study the Bambara groundnut flour samples. Suspension of flour and distilled water was heated with a constant heating rate of 1.5°C / min within a rotating bowl and a measuring sensor reaching into the bowl is deflected. The deflection was measured as viscosity over time, i.e. vs. temperature; and the evaluations recorded includes readings at beginning of gelatinization (°C), gelatinization maximum (BU) and gelatinization temperature (°C).

2.6 Maturograph Evaluation

The Maturograph was used to determine the proving properties of the dough. In a temperature and humidity controlled cabinet the various phases of maturation are recorded automatically. Maturation or fermentation rest is the subjection to fermentation for a suitable length of time to obtain light aerated porous structure for dough used in baking and the time required to achieve this is proofing time. Fermentation of dough was achieved by yeast (*Saccaromyces cerevisiae*). Punching of dough in between the fermentation periods was observed to increase gas retaining capacity of the dough. Dough samples were prepared with a defined mixer (Farinograph®, Do-Corder) at constant consistency of 600 BU. The composition of the prepared dough samples was Bambara groundnut flour (100%), compressed yeast (4%), salt (1.7%), sugar (1.5%), fat (1%) and water needed for the standard dough consistency of 600 BU. Mixing time ranged 3.5–5.0 minutes depending on the dough development and stability, dough temperature was 30°C.

The piece of dough tested (200 g) was placed in the fermentation cabinet of maturograph (temperature 30°C, relative humidity 95%) for a period of 45 minutes. After 20 resp. 10 minutes from the beginning, the dough was remixed in a special maturograph rolling device and after the final handling the piece of dough was placed into the measuring container of maturograph. Maturograph records the fermentation behavior of dough in relation to the proofing time by means of a sensing probe which touches the dough periodically (2 min). The resulting zigzag curve maturogram characterizes the increase of the dough volume and its rheological properties during the proofing time. Thus, final proofing time which is the time in minutes from the start of the final proof to the first drop of the curve after the maximum; proofing stability evaluated in minutes with a stencil in the range of the curve maximum; elasticity which is the band width in the range of the maximum peaks expressed in maturograph units (MU); and dough level which is the value in MU from the zero line to the maximum peak of the curve were measured.

2.7 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 $p < 0.05$. All data are reported as mean of three independent determinations.

3. RESULTS

3.1 Effect of LAB-Fermentation on Functional Properties

The effect of fermentation on the particle size distribution of non-inoculated and inoculated Bambara groundnut were determined as an indicator of flour quality and shown in Fig. 1.

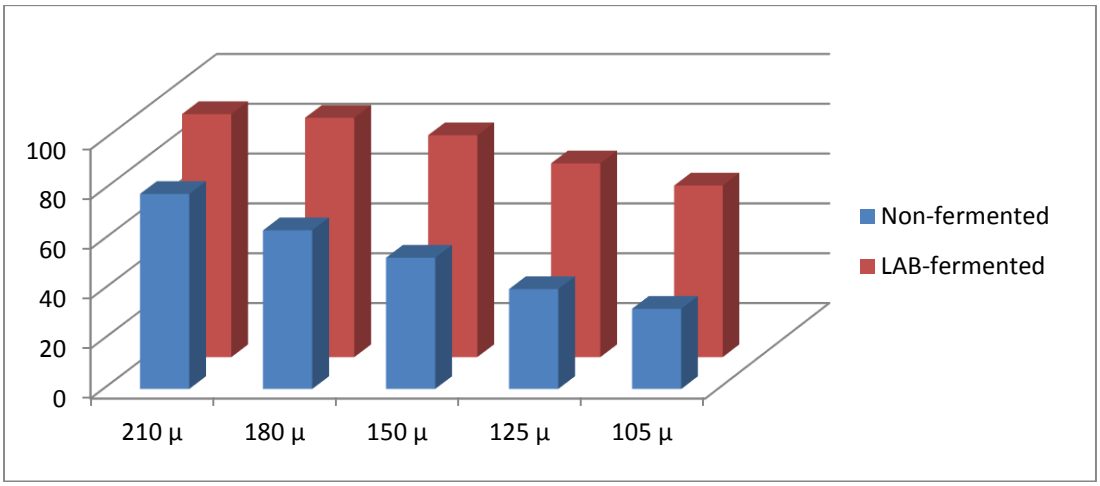

Fig. 1. Particle size determination (%) of the flour samples

Table 1. Functional properties of the flour samples

Parameter (%)	**Non-fermented**	**LAB-fermented**
Water Absorption Capacity	346.50 ^{b}±1.40	386.45 ^{a}±0.60
Least Gelation Concentration	5.30 ^{a}±0.50	4.10 ^{a}±0.20
Swelling Capacity	14.97 ^{b}±0.10	23.20^{a} ±0.30

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

Simple sieve method used to determine flour granulation characteristics of the samples by passing through 210, 180, 150, 125, and 105 µ orifice (Ro-Tech), observed that 150, 125, 105 µ orifice did not readily accommodate particles from the non-inoculated samples; while, inoculated samples passed through 150 and 125 µ but did not readily pass through 105 µ orifice. When the overs were weighed, 210 and 180 µ orifice had the highest passage for both flours but was observed higher in the inoculated sample. The results for this analysis indicates that the flour obtained from bioprocessed Bambara groundnut were more finely milled and produced finer particle sized flour. The functional properties of the flour from non-inoculated and inoculated Bambara groundnut are shown in Table 1. The non-inoculated sample had an absorption capacity of 346.5%, while bioprocess significantly ($p < 0.05$) increased the absorption capacity of the flour to 386.4%. The least gelation concentration of the non-inoculated flour was observed to be 5.3%; thus, the inoculated sample had a value of 4.1%.

The result also noted that swelling capacity of the non-inoculated Bambara groundnut flour was 14.9 mg/100 g while the fermentation of the nut significantly ($p < 0.05$) increased the swelling capacity to 23.2 mg/100 g; thus, indicating that the swelling capacity of flours depends on size of particles and types of processing methods or unit operations.

3.2 Effect of LAB-Fermentation on Rheological Properties

The results of rheological investigations for the non-fermented and LAB-fermented Bambara flour are presented in Tables 2 and 3 for amylograph and maturograph evaluations, respectively. The amylograph recorded the gelatinization properties of flour samples suspended in water during heating and the maximum viscosity attained during the test is the results of both the α-amylase activity and the gelatinization behavior of the flour. Values obtained for amylograph evaluation indicate that temperature at start of gelatinization was peak at 63.8 and 63.0^{0}C for non-fermented and LAB-fermented flour with no significant ($p < 0.05$) difference, while temperature at maximum viscosity and maximum viscosity had significant ($p < 0.05$) values of 92.1 and 76.0^{0}C, as well as 730 and 265 brabender units (BU), respectively. Thus, amylograph quality of the fermented flour sample was indicated by the maximum viscosity which is significantly higher in the case of non-inoculated flour sample. The maturograph evaluation also recorded the maturation behavior

of the dough prepared from the test flours after the proofing time (fermentation rest) by means of a sensing probe which records the elasticity of the mature dough every 2 min and produces the typical zigzag form of the maturogram. The curve rises until maximum dough maturity is reached and drops thereafter. This action was recorded in maturograph units (MU) on the strip-chart with values of 44 and 28 min for final proof time, 750 and 610 MU for dough level, 210 and 220 MU for dough elasticity, as well as 10 and 12 min for proofing stability, determined respectively for non-fermented and LAB-fermented flour samples.

4. DISCUSSION

Particle size distribution and measurement has been used as a direct measure of quality for baking flour and has resulted in the development of several techniques and commercial instruments for this measurement. Sieving, sedimentation [24] and photoextinction technique [25] have received most of the attention in the measurement of particle size distribution of flour. While these procedures are suitable for use with many materials, flour offers some difficulty in analysis because of its shape irregularity and the density differences among its components. Results from this study show that inoculated flour sample show finer particle index than the non-inoculated sample with an average mean value of 78.30 and 97.60 through orifice of 210µ for non-inoculated and inoculated samples, respectively.

The water absorption capacity which is an index of the flour functional quality; is that maximum amount of water that it can take up and retain; hence determine its energy and nutrient dense. Bambara groundnut is a food considered rich in carbohydrates and protein, whereas starch and proteins are the major components of legumes governing their functional properties. A study [26] reported that water absorption capacity is attributed to the protein of the food material. High WAC of the bioprocessed flour suggests that it can find application in baked products. Increase in WAC has always been associated with increase in the amylose leaching and solubility and loss of starch crystalline structure. Flour with high water absorption may have more hydrophilic constituents such as polysaccharides. Protein has both hydrophilic and hydrophobic nature and therefore can interact with water in foods.

The least gelation concentration of the non-inoculated flour was observed to be 5.3%; thus, the inoculated sample had a value of 4.1%. A high gelation value will imply that the flour would require more energy to cook and the gel strength of the diets would be weak and undesirable [27]. Legume flours contain high protein and starch content and the gelation capacity of flours is influenced by physical competition for water between protein gelation and starch gelatinization [28], hence, the lower the LGC, the better the gelating ability of the protein ingredient and the swelling ability of the flour. The present study also observed that fermentation could be used to improve swelling power of Bambara groundnut flour by improving the particle size distribution. High swelling capacity is an important criterion for good quality flour. Since the swelling capacity of the fermented flour is high, it suggests it may find application in noodle production and other pastry foods.

Table 2. Average values of amylograph evaluation

Parameter	Temperature at start of gelatinization (^{0}C)		Temperature at max. viscosity (^{0}C)		Max. Viscosity (BU)*	
	NBG	FBG	NBG	FBG	NBG	FBG
Average	61.0±0.1^a	62.0±0.1^a	88.5±0.4^a	74.5±0.1^b	535±0.2^a	250±0.3^b
Min.	58.2±0.3^a	61.0±0.2^a	85.0±0.4^a	73.0±.3^b	340±0.1^a	235±0.1^b
Max.	63.8±0.5^a	63.0±0.7^a	92.1±0.3^a	76.0±0.9^b	730±1.0^a	265±0.5^b

*Values are means ± SD (n = 3). Values on the same row with different superscript are significantly ($p < 0.05$) different, *BU: Brabender Unit, NBG: Non-fermented Bambara Groundnut, FBG: LAB-fermented Bambara Groundnut*

Table 3. Average values of maturograph evaluation

Parameter	Final Proof Time (min)		Dough Level (MU)*		Dough Elasticity (MU)*		Proofing Stability (min)	
	NBG	FBG	NBG	FBG	NBG	FBG	NBG	FBG
Average	38±0.4^a	25.5±0.1^b	617.5±0.3^a	520±0.6^b	135±0.3^b	195±0.1^a	7±0.2^a	10±0.8^a
Min.	32±0.1^a	23±0.2^b	485±0.4^a	430±0.7^b	160±0.3^b	170±0.1^a	4±0.2^a	8±0.1^a
Max.	44±0.3^a	28±0.4^b	750±0.5^a	610±0.2^b	210±0.2^b	220±0.4^a	10±0.1^a	12±0.4^a

Values are means ± SD (n = 3). Values on the same row with different superscript are significantly ($p < 0.05$) different
**MU: Maturograph Unit*
NBG: Non-fermented Bambara Groundnut
FBG: LAB-fermented Bambara Groundnut

In essence, results obtained for rheological investigation could be used to determine the baking properties of flour depending on the gelatinization of starch and the enzyme activity (α-amylase). This will enable the assessment of the flour quality, suitability of the flour for various applications, measurement of the baking characteristics of the flour, and as well, control of enzyme addition. The result for maturograph analysis showed that proofing time was found to be significantly different, with the LAB-fermented flour sample having a lesser proofing time (range 23-28 min) and the non-fermented flour having a higher proofing time (range 32-44 min). The dough level describing the dough resistance against mechanical stress was slightly lower in LAB-fermented flour sample; while dough firmness (expressed as elasticity) was similar for fermented and non-fermented flour. Thus, proof-ing stability that reflects the time tolerance of optimal proofing and ensures the highest volume of the final product was observed to be higher in fermented and slightly lower in non-fermented samples. Hence, a better unit operation for the flour production may further improve the functionality of the bioprocessed flour.

5. CONCLUSION

In many developing countries of the world, virtually all the wheat used for baking in confectionery industries especially biscuits, bread and cakes are imported. Therefore, campaign on the use of composite flour is best advocated. With the increasing ban on importation in most of these countries, it is hoped that indigenous crops should be revived and used as substitutes for imported ones. Not only could such flour be used in various food applications to improve food security; but could also serve as a protein source in regions were protein from animal source is scarce or expensive. Owing to the outstanding potentials of Bambara groundnut in helping to curb food security issues, this study has demonstrated that the bioprocess of fermentation showed improved flour functionality to all the parameters studied and this suggests that microbial fermentation may offer the simplest means to optimize its utilization as demonstrated in the food products developed. Finding new ways to utilize this legume will obviously increase its cultivation and consumption. Hence, microbial fermentation has demonstrated to improve the functionality of Bambara groundnut and the production processes could be further controlled to get products of optimal quality. This may be achieved by manipulating environmental factors such as temperature, moisture content, aeration, pH, acidity etc., which influence the activity of microorganisms during the fermentation process.

DISCLAIMER

There is absolutely no conflict of interest between the authors and owners of the starter culture used for this study because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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