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Microbial Lactic Acid Fermentation Improves Nutritional and Organoleptic Profile of Non-Dairy Milk Made from Bambara Groundnut

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ABSTRACT

Non-dairy milk was produced from bioprocessed Bambara groundnut 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). Bambara groundnut was submerged in sterile water, inoculated with the starter culture containing 10^6 CFU/mL, and allowed to stand for 3 days. Bambara milk was produced by wet-milling the nut and the resulting paste cooked on medium heat for 20 min and strained with cheesecloth to remove the particles. Milk from non-inoculated Bambara nut and cow milk was used as positive and negative controls for sensory characteristics and nutritional comparison. The nutritional and sensory profile was compared to meet WHO recommended daily intake (RDA) with cow milk. Proximate composition of the samples ranged from 20.80 - 19.70, 57.20 - 52.25, 6.80 - 8.79% and 368.10 - 425.10 Kcal/100g for protein, carbohydrate, fat and energy, respectively. Results show that protein content of the fermented Bambara milk (19.70) was higher than cow milk (3.4) while fat content (8.79) was higher compared to cow milk (3.6 g/100g). Amino acids content ranged from 3.90 - 5.00, 9.00 - 14.20, 5.62 - 6.80, 0.6 – 0.92, 17.20 – 19.50, 3.35 – 3.80, 2.50 – 3.00, 3.80 – 4.15, 7.00 – 8.00, 2.90 – 4.50, 2.80 – 3.20, 4.80 – 5.10, 3.80 – 4.50, 2.60 – 3.80, 2.60 – 4.00, 3.50 – 3.90 and 4.10 – 4.85 for alanine, arginine, aspartic acid, cystine, glutamic acid, histidine, isoluecine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine, respectively. In-vitro protein digestibility increased from 70.74 - 89.70%, while anti-nutritional factors decreased from 4.72 – 2.08, 870.30 – 383.70, 1470.15 – 1023.10 and 1.85 – 0.55 mg/100g for tannin, polyphenol, phytate, oxalate and trypsin inhibition activity, respectively. Intensities of the sensory attributes was assessed based on parameters of appearance, aroma, mouth feel, consistency and overall acceptability using a nine-point Hedonic scale rating, and the results found to compare favorably with cow milk.

INTRODUCTION

With the increasing demand for alternative protein sources and healthier less processed foods, the food process industry is constantly searching for nutritious and consumer acceptable plant-based foods to meet the nutritional needs of various age groups. Bambara groundnut (*Vigna subterranean* (L)) is a legume crop in the sub-Saharan Africa grown mainly by subsistence farmers. It is indigenous to West and Central Africa and considered a highly nutritive but underutilized grain legume. Our recent study (Chude *et al*., 2021) 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. Previous studies noted that Bambara grains contains 54.5 - 69.3% carbohydrate, 17 - 24.6% protein, 5.3 - 7.8% oil, and the gross energy value is greater than that of other common pulses like pigeon pea (Cajanus cajan) and cowpea, lentil (Lens esculenta) (Azam-Ali *et al*., 2001; Quaye & Kanda, 2004). Despite less interest in the commercial production of the crop and the almost total scientific neglect to improve yield, Bambara groundnut greatly contributes to the dietary structure of many parts of Africa (National Research Council, 2006). In addition to its commendable nutritional composition, Bambara groundnut possesses outstanding traits for drought tolerance, nitrogen fixation, and ability to produce yields in marginal soils, among others. Improved Bambara groundnut cultivars do not exist (Linnemann & Azam-Ali, 1993); it exists as landraces, which actually composed of many genotypes that is reported to result in an ability to endure stresses under local agricultural systems (Zeven, 1998).

Lack of adequate processing techniques is said to hinder the utilization of this legume crop which has limited its production. Fermentation using lactic acid bacteria (LAB) provides a good measure to improve its hard-to-cook phenomenon and optimize its utilization through improved palatability and nutrient availability (Chude *et al*., 2018a, b; Masood *et al*., 2011). LAB fermentation has been used to improve preservative and detoxifying effects on fermented food product (Day & Morawicki, 2018). The process of LAB fermentation also provides a cheap processing technique that has the potential to improve the digestibility of protein in grains and increase the protein content (Chude *et al*., 2021). Bambara groundnut like many underutilized crops in the African region has the potential to improve food security measures and also contribute to problems of insufficient protein availability

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which is a major problem in many developing countries due to the high cost of protein from animal sources. Alternative measures to improve protein availability through other sources than animal protein becomes necessary to alleviate this problem.

Lots of work has been done on the production and nutritional status of non-dairy plant-based milks like soy milk, almond milk, coconut milk, rice milk or hemp milk. However, Bambara groundnut also has the potential of a milk substitute that can be utilized by individuals intending to avoid products of animal origin for ethical or health reasons, or simply for taste preferences. Most food producers market plant-based milk substitutes as being healthier than cow milk because they may be lower in saturated fat and, if entirely free of animal products, cholesterol-free.

Cow milk related health problems such as lactose intolerance in persons deficient in the enzyme lactase which is responsible for breaking down the lactose in the intestine can be solved with non-diary milk alternatives. Health symptoms such as abdominal cramps, constipation, bloating or diarrhea may result in lactose intolerant individuals who consume a dairy product. Amongst infants and children, dairy milk is considered common allergens although many tend to outgrow it in later years. More so, dairy milk especially unpasteurized milk is considered to have food safety concerns and has been linked to outbreaks of food pathogens around the world such as *Salmonella and E. coli* outbreaks. Owing to the outstanding qualities of Bambara grains, there is need to research novel ways that can be used to effectively harness its use and potentials in various food product applications. Hence, the study aims to evaluate the nutritional and sensory properties of non-diary milk produced from fermented Bambara grains.

MATERIALS AND METHODS

Our previous studies (Chude *et al*., 2021; Chude *et al*., 2018a, b) described the pre-handling operations of the Bambara groundnut and LAB starter culture used in the fermentation process. Briefly, the nuts were carefully cleaned and all extraneous materials and damaged nuts removed prior to use. Washing of the nuts was done twice using ordinary water while rinsing was done with distilled water prior to cooking to softness as a pretreatment measure and to eliminate existing microflora before starter cultures inoculation. *Lactobacillus plantarum* [NRRL B-4306] and *Lactobacillus fermentum* [NRRL B-1932] used in this experiment were obtained from USDA Agricultural Research Services Culture Collection as pure cultures of freeze dried cells preserved in a dormant state. Inoculation in 25 ml Nutrient Broth and incubated in CO_2 enriched jars for 24 h was used to bring the freeze dried cells to active state; cell recovery was achieved by centrifugation at 3600-x g for 15 min.

The recovered cells were rinsed using 10 ml sterile distilled water and the spine repeated twice. Afterwards, a suspension of the cells in 9 ml sterile distilled water was made and serially diluted before plating on Plate Count Agar using pour plating method. The colonies on each plate of the dilution factor were counted after 24 h incubation in CO_2 enriched jars and only plates containing approximately 10^6 cfu/ml was utilized in the inoculation of the fermentation process.

Production of Bambara Milk

The pre-treated Bambara groundnut used in this experiment for the production of Bambara milk was submerged in 30 liters of sterile distilled water and inoculated with 10^6 cfu/ml of the *Lactobacillus plantarum* [NRRL B-4306] and *Lactobacillus fermentum* [NRRL B-1932] obtained from USDA. Fermentation was allowed for 3 days after which the LAB-fermented Bambara groundnut and control was wet-milled. The resulting paste was poured into a pot, cooked on medium heat for 15 min, and stirred continuously so it does not stick to the bottom of the pot. After 15 min, the foam was scooped off and the samples allowed to cool down to about 100C. Then cheesecloth was used to strain the samples to remove the particles, and it was pasteurized at low heat for 5 min, poured into a bottle, refrigerated and analyzed within 12 hours of production. Milk from non-inoculated Bambara groundnut and Cow milk was used as control for sensory characteristics and nutritional comparison.

Determination of Proximate Composition and Total Energy

Standard procedures described by the Association of Official Analytical Chemists (1990) were used to determine the nutrient composition of the samples. Briefly, the Kjeldahl method was used for protein (N × 6.25) determination while soxhlet extraction with a known weight of sample in petroleum ether (boiling point, 40 to 60°C) was used for crude fat determination. The carbohydrate content was determined by sample differences of subtracting the total fat, crude protein, ash, and crude fiber from the total dry weight (100g) of the sample. A ballistic bomb calorimeter and the caloric value estimation was used for the gross energy determination according to Antia *et al*. (2006) by summing the multiplied values for crude protein, oil, and carbohydrate by their respective factors.

Determination of Mineral Content

The dry ashing method originally described by Chapman and Pratt (1982) was used for minerals determination. Sample size of 2 g was acid-digested with diacid mixture (HNO_3:$HClO_4$, 5:1, v/v) in a digestion chamber and the digested samples 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 were determined spectrophotometrically using molybdovanadate method.

Determination of amino acids

The amino acids compositions of the samples were measured on protein hydrolysates based on high performance liquid chromatography technique according to the method described by Chude & Nkama (2020). The fermented and non-fermented Bambara milk samples were weighed into conical flasks with 100 mL of 70 % methanol. The mixture was shaken for 5 min using a mechanical rotor and allowed to stand for 10 min. The resultant supernatant was filtered through Whatman #1 filter papers and diluted with deionized water in a volume ratio of 1:3; and the pH adjusted to a range of 5-8 using HCl and NaOH as described by Jayaratne *et al.* (2020). The filtered samples will be utilized for HPLC following protocol outlined by the manufacture. A standard solution containing 1.25 µmol/mL of each amino acid in 0.1N hydrochloric acid was created.

Derivatization of amino acids were done using a standard solution (20 µL) pipetted into a 10 × 5-mm tube and dried in vacuo at 65°C. The residue was added 30 µL of methanol-water-Phenylisothiocianate (2:2:1 [v/v]) and then removed in vacuo at 65°C, after which 30 µL of the derivatizing reagent methanol-water-Phenylisothiocianate (7:1:1:1 [v/v]) was added, and the tube was vortexed for 30 sec and allowed to stand for 20 min at ambient temperature. Aliquot of 150 µL of the diluent containing 5mM sodium phosphate with 5% acetonitrile was added to each tube before injection. Chromatographic analysis was obtained at 30°C using a gradient elution (Table 1). An aqueous buffer (Eluant A) was prepared using 0.5 mL/L Triethylamine added to 0.14M sodium acetate and titrated to pH 6.20 with glacial acetic acid while eluant B was acetonitrile-water (60:40 [v/v]). Each amino acid was calculated based on the proportional molar concentration using the concentration of standard amino acids and expressed as µ g amino acid/mg sample.

Table 1: Gradient program employed for the separation of PTC-amino acids

Time	**Flow rate**		
(min)	**(mL/min)**	**% Eluent A**	**% Eluent B**
0	1.0	90	10
12.0	1.0	70	30
20.0	1.0	52	48
22.0	1.0	0	100
24.0	1.0	0	100
30.0	1.5	0	100
37.0	1.0	90	10

Determination of anti-nutrients Composition

Determination of Tannin Content

The modified vanillin-HCl method originally described by Price *et al.* (1978) was used for the quantitation of tannins in the test and control samples. Briefly, 200 mL of the samples were extracted for 20 min in capped rotating test tubes using 10 mL 1% (v/v) conc. HCl in methanol. Aliquot of 0.5% of 5 ml vanillin reagent was added to the extract (1 ml) and the absorbance of the color determined at 30°C after 20 min and the absorbance read at 500 nm. An interference natural light pigment was corrected in the sample by subjecting the extract to same conditions of the reaction without the vanillin reagent. The results were expressed as catechin equivalents using standard curve, i.e amount of catechin (mg per ml) which gives a color intensity equivalent to that given by tannins after correcting for blank.

Determination of Phytic acid Content

Following methods originally described by Wheeler & Ferrel (1971), 3 mL of the samples were used for extraction of phytic acid using 3% trichloro-acetic acid kept on a vortex shaker at ambient temperature and subsequently centrifuged on high speed. Precipitation was used to obtain the phytic acid from the supernatant by estimating the ferric phytate and iron in the samples. Thus, a 4:6 iron: phosphorus molecular ratio was used to calculate the Phytate-phosphorus (phytate-P) from the iron determined. The phytic acid was then estimated by multiplying the amount of phytate-phosphorus by the factor 3.55 based on the empirical formula $C_6P_6O_{24}H_{18}$.

Determination of Oxalate Content

The AOAC (2010) method was utilized for oxalate determination. To this regard, 1 g each of the samples was weighed into 100 ml conical flask and was added 75 ml of 3 M H_2SO_4. The mixture was intermittently stirred for about 1 hour with the aid of a magnetic stirrer and then filtered using whatman No.1 filter paper. The filtrate was adjusted to 25 ml and titrated at 80 - 90°C using a solution of 0.1 N KMnO4 until a faint pink colour that persisted for at least 30 sec appeared. The oxalate concentration in each sample was obtained from the calculation: 1 ml 0.1 permanganate = 0.006303 g oxalate.

Determination of Polyphenol Content

The spectrophotometric method originally described by Price and Butler (1977) was utilized for polyphenols quantification using sample size of 50 ml mixed with 3 ml of methanol in a test tube and was manually shaken for 60 sec. The mixture was filtered using whatman No.1 and the filtrate mixed with 50 ml of water. Analysis of

the samples was done within an hour. For the analysis, 3 ml aliquot of 0.1 M $FeCl_3$ in 0.1 N HCl was added to 1.0 ml of the filtrate and timed addition of 3 ml of 0.008 M $K_3Fe(CN)_6$ was done. The absorbance was read at 720 nm after 10 min using a spectrophotometer. A standard curve was prepared using tannic acid and following the above procedure.

Determination of Trypsin Inhibition Activity

The inhibitory action of the bovine trypsin (EC 3.4.21.4) on substrate benzoyl-DL-arginine-p-nitrianilide (BAPNA) hydrochloric was used to determine trypsin inhibition activity (Kakade *et al.*, 1974). The samples (1 ml each) were extracted for 3 hours with 50 ml, 10 M NaOH using a mechanical shaker continuously at ambient temperature. The pH of the resulting solution was adjusted to 9.4 - 9.6 with 1 M NaOH and the suspension was shaken and diluted with distilled water to 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).

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

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 in-vitro Protein Digestibility

Measurement of in-vitro protein digestibility of the samples was determined according to the method originally described by Maliwal (1983). In triplicate, a known quantity of the test and control samples was mixed with 16 mg nitrogen and digested in 15 ml of 0.1 M HCl for 2 h at 37ºC using 1 mg pepsin. Aliquot of 15 ml of 10% trichloroacetic acid (TCA) was added to stop the reaction. Centrifugation of the mixture was done at 630 rev. for 5 min and the resultant filtrate passed through Whatman No. 1 filter paper for quantification. Thus, the TCA soluble fraction was assayed for nitrogen using the micro-Kjeldahl method (AOAC, 2010). The following equation was used to obtain the digestibility of the samples:

$$\text{Protein digestibility (\%)} = \frac{\text{N in Supernatant - N in blank}}{\text{N in Sample}} \times 100$$

Determination of Sensory Characteristics

Sensory qualities of the test and control products were conducted with the 10 member panelists trained to identify retronasal aroma and taste to determine sensory attributes. A consent form was given to the panelists and was also educated on their duties. A randomized, single-blind manner was employed for the evaluations were panelists were blinded from the identity of the specific product to be consumed. The product was labeled prior to the start of experiment with a randomization code and the code list kept confidential from the panelist during the experiment. The test was conducted while the samples were still fresh and the panelists were required to observe the sample, taste and score; then rinse their mouth with water before tasting another sample/product.

As described by Arteaga *et al.* (2021), 20 mL of each sample presented in random order at ambient temperature in glass cups sensory analysis was done using. The sensory analysis was divided into two sessions for presentations of fermented / unfermented Bambara milk sample and fermented / cow milk samples per session. For palate cleansing, water and plain crackers were provided. The panelists assessed the intensities of the attributes based on parameters of appearance, aroma, mouth feel, consistency and overall acceptability using a nine-point Hedonic scale rating of 9 = liked extremely down to 1 = disliked extremely.

Data Analysis

Analysis of the data generated from the experiment was conducted in triplicates and expressed as mean ± SD using Tukey–Kramer multiple comparisons test or two-way analysis of variance (ANOVA). Pair-wise statistical comparison and the relevance between samples was evaluated using the Mann-Whitney rank sum test in SigmaPlot (Systat Software, USA). Statistical difference between fermented and non-fermented Bambara milk was evaluated using the Student's T-test. P-value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Table 2 presents the proximate composition and total energy of the test and control Bambara milk samples in comparison to recommended daily intake of adults. The protein content of non-inoculated Bambara milk was found to be 20.80% which was similar to that reported by Abdulsalami & Sheriff (2010) but higher than that reported by Okonkwo & Opara (2010) for raw Bambara seed. The protein content of the Bambara milk slightly decreased after fermentation (19.70%). The result indicates that bioprocessing of the seeds had no significant effect on protein content. However, an increment in protein content after soaking was observed by Hassan *et al.* (2005) for lupin seeds and explained that the increment resulted due 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). Similar reduction in protein after cooking was observed by Hamed *et al.* (2008) and Hainida *et al.* (2008) for pumpkin and roselle seeds, respectively.

Table 2: Proximate composition, mineral content and total energy of samples

Paramet+1:10ers	NBM	IBM	Cow Milk	RDI*
Protein (g/100g)	20.80±0.8%[a]	19.70±0.2%[a]	3.4. ± 0.9	50g
Fat (g/100g)	6.80±0.1%[a]	8.79±0.5%[b]	3.6. ± 0.2	Less 70g
Carbohydrate (g/100g)	57.20±0.4%[a]	52.25±0.7%[b]	53.40 ± 0.2	Least 260g
Mn (mg/100 g)	3.00 c±0.60	1.90 d±0.40	11.7 ± 1.0	2.0mg
P (mg/100 g)	265.80 a±0.40	248.40 b±0.60	93.5 ± 0.4	1,000.00mg
Ca (mg/100 g)	220.30 a±0.80	198.80 b±0.2012	6.4 ± 0.5	1,000.00mg
K (mg/100 g)	50.20 c±0.90	38.90 d±0.20	138.5 ± 0.1	3,500.00mg
Na (mg/100 g)	11.50 c±0.50	7.80 d±1.00	58.2 ± 1.0	2300mg
Energy (Kcal/100g)	368.10±1.0[b]	425.10±1.5[a]	252.58 ± 0.01	2,000Kcal

*Values are means ± SD (n = 3). Values in the same row with different superscripts are significantly (p < 0.05) different. NBM: Non-inoculated Bambara Milk, IBM: Inoculated Bambara Milk*Recommended Daily Intake (RDI) of Adults (WHO, 1996; NIH, 2020)*

The fat content of non-inoculated milk was 6.80% and was similar to that reported by Abdulsalami & Sheriff (2010) and higher than that of Mune *et al.* (2007) for raw Bambara seeds. Bioprocess significantly increased fat content of the Bambara milk (P<0.05). Bradbury *et al.* (1984) 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 increase in crude fat content of sorghum and sorghum plus green gram blend during natural fermentation. More so, the carbohydrate content of non-inoculated sample was found to be 57.20% and was similar to that reported by Okonkwo and Opara (2010) for raw Bambara seed. The carbohydrate content significantly (P>0.05) decreased after fermentation. The changes observed are possibly due to leaching of soluble components into cooking (Yagoub & 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. The gross energy content significantly (P<0.05) increased with a maximum value of 425.10 kCal/100g. The calculated metabolizable energy values which ranged between 368.10 and 425.10 kCal/100 g showed that Bambara groundnut have energy concentrations favorably comparable to cow milk.

Determination of mineral content indicates that the non-inoculated Bambara milk was found to be rich in calcium. Calcium content of flour from non-inoculated nuts was 220.30 mg/100 g which decreased to 198.80 mg/100 g after fermentation. The results indicated that 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. 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 (Table 2). 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 also 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. However, the mineral content of the inoculated Bambara milk despite the decrease was found to compare favorably with cow milk and with the RDA of WHO (1996).

Figures 1 and 2 shows the chromatogram for amino acid analysis of the test (fermented) and control (non-fermented) samples. The study for amino acid composition observed that glutamic acid, aspartic acid and leucine are the most abundant amino acids in Bambara milk. Amino acid contents slightly increased after fermentation. Similar observation has been reported by Olaofe & Akintayo (2000), and Adeyeye & Afolabi (2004) for soaking and cooking of Bambara groundnut; glutamic acid was the most concentrated essential amino acid (17.00%). Other researchers 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 & Bisping (1995) found that certain Rhizopus strains with high proteolytic activity were able to release nearly 5 times more amino acids. 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. Ferial & Esmat (2011) observed 21.8% increases in essential amino acids in fermented chickpea with Rhizopus at 48 hours of fermentation. Thus, when comparing the essential amino acids in Bambara milk with the recommended FAO/WHO provisional pattern, it was superior with respect to aspartic acid, threonine, methionine, leucine, tyrosine, phenylalanine, histidine and arginine and adequate in valine and isoleucine (Table 3), and it was only for lysine that supplementation might be required.

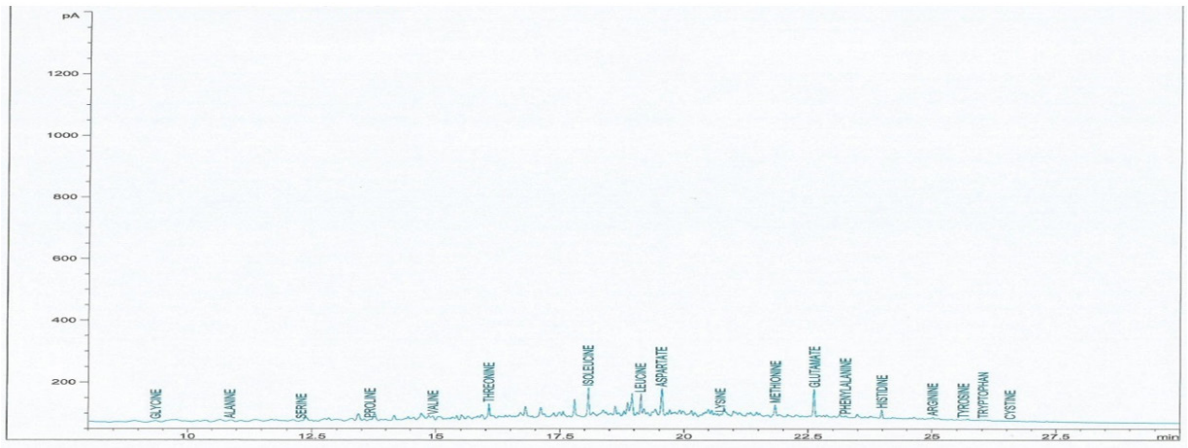


Figure 1: HPLC Chromatogram of LAB-fermented Bambara groundnut

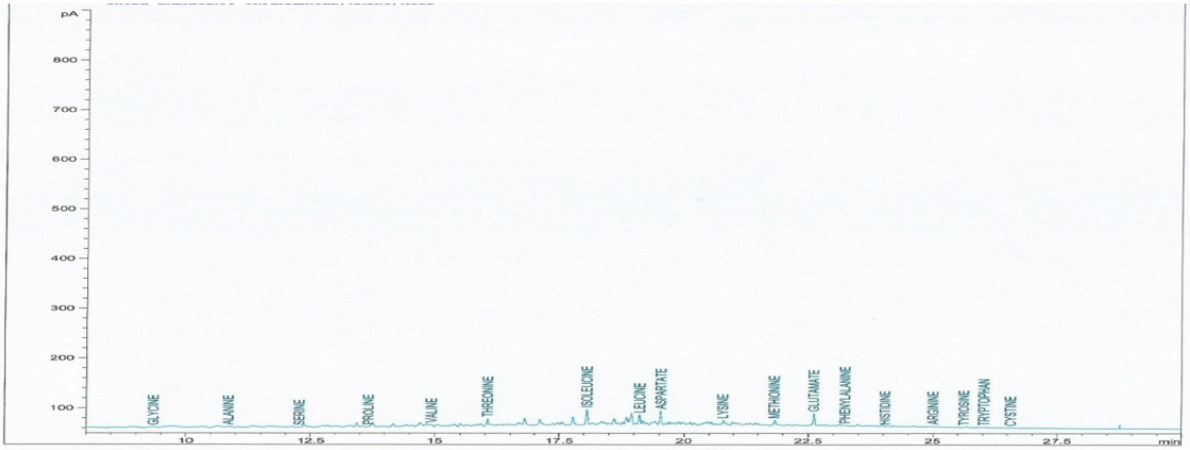


Figure 2: HPLC Chromatogram of non-fermented Bambara groundnut

Table 3: Amino acid composition of samples

Amino Acids	NBM	IBM	Cow Milk*	Reference Daily
Alanine	3.9	5.0	4.15	
Arginine	5.0	6.2	2.9 – 4.2	2.0
Aspartic Acid	5.62	6.8	6.2 – 7.8	4.0
Cystine	0.6	0.92	0.65	
Glutamic Acid	17.2	19.5	15.8 – 23.2	
Glycine	3.35	3.8	0.8 – 2.1	
Histidine	2.5	3.0	3.0	2.4
Isoleucine	3.8	4.15	4.1 – 6.2	4.2
Leucine	7.0	8.0	3.2 – 8.3	4.8
Lysine	2.9	4.5	8.1	4.2
Methionine	2.8	3.2	3.2	2.2
Phenylalanine	4.8	5.1	5.4	2.8
Proline	3.8	4.5	10.1 – 11.8	
Serine	2.6	3.8	6.6	
Threonine	2.6	4.0	5.8	2.6
Tyrosine	3.5	3.9	5.8	1.4
Valine	4.1	4.85	7.5	4.2

Values are mean of triplicates. NBM: Non-inoculated Bambara Milk. IBM: Inoculated Bambara Milk.
** Saima et al. (2016)* *WHO (1996)*

Table 4: Antinutritional factors and in-vitro protein digestibility of samples

Antinutrients	NBM	IBM	Daily Reference Intake*
Tannin (mg/100 g)	4.72±1.0[a]	2.08±0.2[b]	1.5–2.5 g
Polyphenols (mg/100 g)	870.30±0.3[a]	383.70±0.5[b]	500mg to 1500mg
Phytic acid (mg/100 g)	1470.15±0.5[a]	1023.10±0.1[b]	100-400 mg
Oxalate (mg/100 gm)	1.85±0.8[a]	0.55±0.5[b]	50 – 100 mg
Trypsin Inhibitor (mg/100 g)	8.40±0.2[a]	3.30±0.4[b]	-----
IVPD (%)	70.74±1.0[b]	89.70±0.6[a]	-----

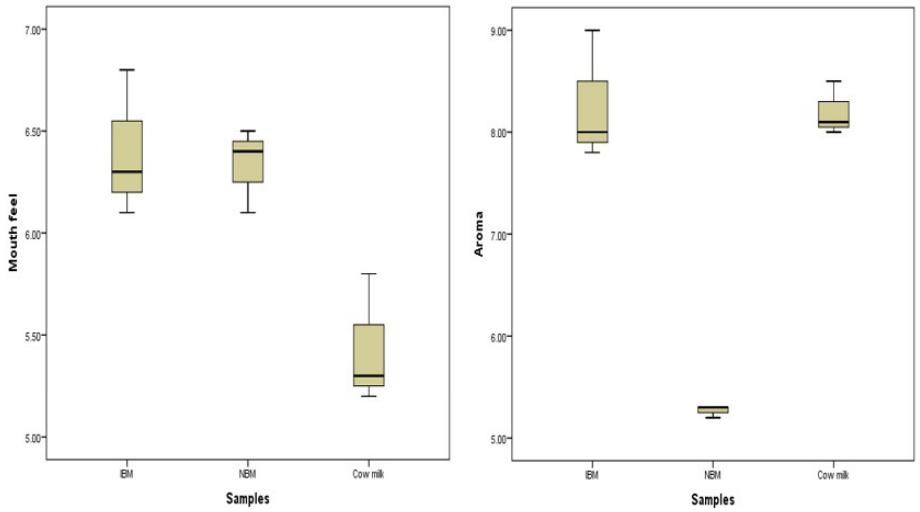
*Values are means ± SD (n = 3). Values on the same row with different superscripts are significantly (p < 0.05) different. NBM: Non-inoculated Bambara Milk, IBM: Inoculated Bambara Milk. *(Finkielstein and Goldfarb, 2006; Taguchi et al. 2015; Raj et al. 2015; Sharma et al. 2019)*

The antinutritional factors of the samples are shown in Table 4. Tannin content of the non-inoculated Bambara milk (4.72 mg/100g) was higher than that reported by Abiodun & Adepeju (2011) for Bambara groundnut. Fermentation significantly (P > 0.05) decreased tannin content to 2.08 mg/100g. Similar trends 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. Polyphenol content of non-inoculated sample was 870.30 mg/100g, and significantly (p > 0.05) decreased after fermentation to 383.70 mg/100g. These results were in agreement with the findings of Yagoup *et al.* (2004) for roselle seeds. The phytate content also had a similar trend of decrease. Bambara groundnut is rich in protein; therefore they had high phytate levels. In legumes, phytates are associated with protein bodies (Sulieman *et al.*, 2007) and, therefore, phytate levels should increase with increasing protein content.

These results revealed that fermentation could lower the level of these antinutrients. The loss in phytates during fermentation of Bambara groundnut may be due to leaching of phytate ions into the 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 the soaked bean have been earlier reported (Bishnoi *et al.*, 1994). Fermentation also significantly (p > 0.05) decreased oxalate content to 0.55 mg/100g; while trypsin inhibition activity has a content of 8.40 mg/100g for non-inoculated sample against 3.30 mg/100g observed after fermentation.

Table 4 also presents the in-vitro protein digestibility of the test and control samples. The increment in protein digestibility after fermentation is likely due to reduction in antinutrients as a result fermentation. Effective reduction of antinutrients has been reported to improve the protein digestibility in legumes (Babiker and ElTinay, 1993). Legume consumption has been associated to 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). It is evident from the study that antinutrient concentration in legumes can be eliminated or reduced to tolerable level through lactic acid bioprocess.

Sensory attributes were determined to monitor the potentials of the product and ascertain the consumer perception of the fermented Bambara milk in comparison to the non-fermented sample and cow milk, and the data presented in Table 5. Evaluation for color, consistency, mouth feel, taste and overall acceptability showed that Bambara milk can compete favorably with cow milk in consumer acceptability. If a new product or formulation fails to appeal to consumers, the new product is considered as an unsuccessful development.



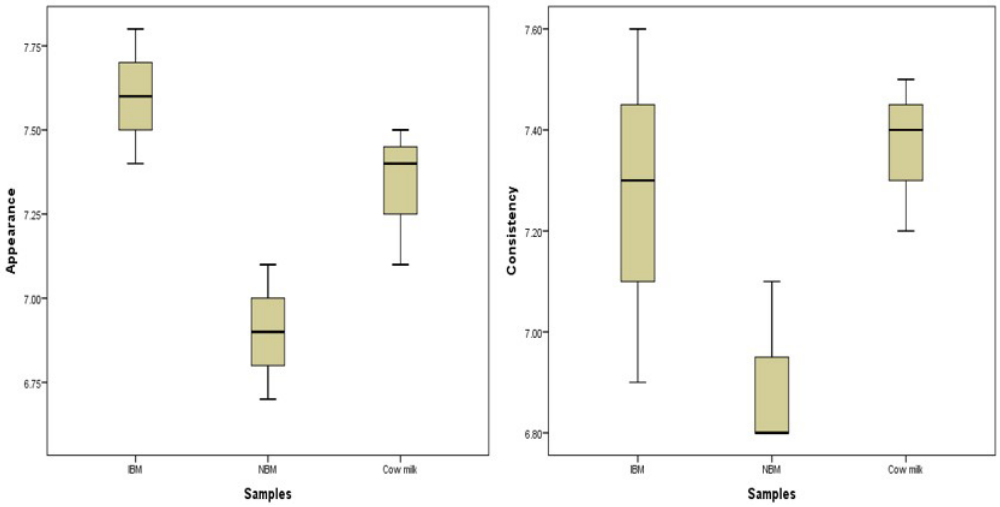


Figure 1: Box plot illustrating sensory evaluation of fermented Bambara milk and control

The result for color showed that fermented Bambara-milk had a mean score of 8.3, which was extremely liked. Color is considered an important property of food product and could affect consumer acceptance of the product. The Bambara-milk had a pale yellow color and contained no artificial color additive. The mean value for consistence was shown to be 7.7 and had no significant ($p > 0.05$) difference, when compared to values of 7.4 and 7.8 obtained for non-inoculated milk and cow milk respectively. Consistency is an important attribute for beverages and in commercial beverage products; emulsifiers and stabilizers, such as guar gum, κ-carrageenan and xanthan gum, may be added to improve the quality of the product by promoting its thickness. Hence, the result obtained for consistency of the product indicates that this will not be needed in commercializing Bambara-milk as the product was liked without artificial emulsifiers and stabilizers.

Bioprocessed Bambara milk had a mean score of 7.8 for taste and no significant ($P > 0.05$) difference with the control sample. It was observed that the fermented sample had no beany flavor ordinarily observed in products made from Bambara groundnut. This was attributed to the actions of the lactic acid bacteria used in the fermentation process. It could also be possible that the fermented Bambara nuts used in the production was lipoxygenase free through fermentation. According to Yuan and Chang (2007), the beany flavor components are mainly the oxidation products of unsaturated lipids catalyzed by lipoxygenases. When no lipoxygenase exists in legumes, polyunsaturated lipids will not be catalyzed to produce the undesirable flavor. Mouth feel was used as an indication of texture for the Bambara milk and characterized by degree of hardness or softness of the product. The sensorial data obtained for mouth feel had a mean score of 6.7 and no significant ($P > 0.05$) difference with cow milk which was preferred. Hence, mouth feel is an important parameter to consider in developing new beverage products, as this will also influence consumer perception and acceptability.

CONCLUSION

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. This study has shown that milk from this fermented legume can compare favorably with cow milk with regards to consumer acceptability as well as the recommended FAO/WHO nutritional provisional pattern. This will provide a good alternative for consumers who wish to avoid intake of animal products due to cholesterol content or personal belief. The study also showed that microbial fermentation may offer the simplest and economic means to improve the safety, quality and functionality of Bambara groundnut, hence, optimize its utilization as plant-based milk. In developing regions where population is constantly on the increase, food security becomes paramount and there is no better means of ensuring food security than harnessing the potentials of indigenous crops.

Considering the qualities mentioned for Bambara groundnut, it has great opportunities towards food security, sustainability, income generation, product development, and dietary diversification. Although the crop is limited by the content of anti-nutritional factors, microbial fermentation provides an effective and safe means to biodegrade these anti-nutrients. Owing to the outstanding potentials of Bambara groundnut in helping to curb food security issues, it should not only be seen and cultivated as subsistence crop; rather it should be seen as a crop that is relevant to food security.

However, there is need to further investigate the scope for improvement in fermentation of Bambara groundnut, since most fermented foods are produced at household level in a majority of African countries where this legume is indigenous. Upgrading the production process for fermented Bambara groundnut will necessitate several critical steps in the commercialization of plant-based milk from this important legume crop. Identified microorganisms, which have the ability to effect beneficial

changes in the fermentation process of this legume should be selected and subjected to genetic improvement geared towards maximizing desirable quality attributes and limiting any undesirable attributes such as anti-nutrients and beany-off flavour.

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