The Chemical Composition of Bambara Groundnut Flours at Different Fermentation Periods

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ABSTRACT

Background: *The antinutrients composition in bambara groundnut has predisposed a lot of people to health risks. Therefore, the study to determine the effect of fermentation on the nutrient and antinutrient becomes pertinent.*

Objective*: The study assessed the chemical composition of flours from fermented bambara groundnut.*

Materials and methods: *An experimental study design was used. The bambara groundnuts were fermented at different periods of twenty-four hours (BTF), fourty-eight hours (BFE), seventy-two hours (BST) and the unfermented portion coded zero (BZE) was used as the control. Proximate analysis was done and the mean, standard error of the mean (SEM) and analysis of variance (ANOVA) were done using statistical package for service solutions (SPSS) version 21. Means were separated by Duncan's Multiple Range Test and significance judged at P <0.05.*

Result: *There were no significant differences (P>0.05) in the moisture content. The control (BZE) had the highest carbohydrate (69.63%), fat (1.63%) though not significantly different (P>0.05) and a higher fibre content. Sample BFE had the highest protein value (21.82%) and ash (2.55mg) at a significant level (P<0.05). Sample BST had the lowest fibre value (1.20%). For the minerals assessed, BFE had the highest values in calcium and iron while BTF (24hr) had the highest in magnesium, manganese, copper and zinc. There was no significant difference in the vitamin C, thiamine and riboflavin values. The antinutrient values of BZE was significantly higher than all of the sample flours (P<0.05).*

Conclusion: *The result showed that fermented flours of bambara groundnut had increased nutrients and reduced antinutritional factors.*

Keywords: Bambara Groundnut, Flours, Fermentation Periods, Nutrient, Antinutrient

INTRODUCTION

Bambara groundnut (*Voandzeia subterrenea)* is a highly nutritious crop that plays an important role in people's diet having about 63% carbohydrate, 19% protein and 6.5% oil (1). It is an important legume consumed in Nigeria, especially in Enugu State, some Northern States and Northern Cameroon (2). In Nigeria, the freshly harvested pods of bambara groundnut are either roasted and eaten as snack (3) or milled into flour and used in preparation of moin-moin (4). For most food uses, seeds of legumes including Bambara are removed to reduce antiphysiological factors and fibre content and this results in better appearance, texture, cooking quality, palatability and digestibility of the products (5). Bambara groundnut seeds which are known as 'okpa' in Ibo states of Nigeria are either roasted and chewed with palm kernel as snack item or they may be boiled and eaten as pottage. More commonly, the seeds are milled into flour and used to prepare bean balls ('akara') after frying the paste in vegetable oil or the slurry may be prepared to the popular steamed gel also known as 'okpa' (6). Unfortunately, like other legumes, Bambara groundnut contain some antinutritional factors such as hemagglutinin, cyanide, trypsin inhibitor, tannins and phytate, and negative nutritional factors which include protein and carbohydrate indigestibility, and sulphur amino acid deficiency (7), which limits its utilization. To this effect, although cooking can reduce these antinutrients to a safe level (8) but fermentation offers additional advantages having been utilized in other legumes such as baobab (9), soybeans and sorghum and its effect established.

These problems encountered with legumes made it necessary to use processing methods that will provide improved nutritional quality and acceptability of these plant foods (10). Meanwhile (11) identified fermentation as an economic processing method that could be used in homes to improve the nutritional quality of plant foods like legumes.

Fermentation plays an important role in human nutrition. It is not only an important adjunct to getting nutritional value from foods; it can prolong shelf life and improve the palatability of many foods (12). Fermentation is a desirable process of biochemical modification of primary food matrix brought about by microorganisms and their enzymes (13). Fermentation is used to enhance the bioaccessibility and bioavailability of nutrients from different crops including maize (14) With these desirable benefits, fermentation has been considered as an effective way to reduce the risk of mineral deficiency among populations, especially in developing countries where unrefined cereals and/or pulses are highly consumed (15). Fermentation of legume and indigenous oil seeds like African locust beans (*Parkia biglobosa*), melon and oil bean have resulted to food products such as 'iru' ('dawadawa') produced from fermentation of seeds of African locust beans, 'ogiri' from melon seeds (citrus vulgaris) and 'ugba' from oil bean (16). Meanwhile, much work has not been done on the use of fermentation to produce flours from bambara groundnut for the purpose of utilization it in making food products.

Recently there has been interest in the improvement and utilization of underutilized food crops like potato (sweet), cocoyam and others. This has aroused the enthusiasm to this research. This study therefore was designed to evaluate the nutrient and antinutrient composition of bambara groundnut fermented at different periods of time.

Materials and methods

Healthy and mature seeds of Sokoto white specie of the bambara groundnut (*Voandzeia subterranea*) were purchased from Umuahia Main Market. The seeds of the bambara groundnut used for the study were cleaned by manual sorting or handpicked to remove dirts and stones. Eight hundred grammes (800g) each of the bambara groundnut seeds were steeped in clean water and fermented for 24hrs, 48hrs and 72 hrs. The seeds were allowed to ferment by the natural microflora of the seeds. One portion of the Bambara groundnut seeds was left unfermented.

At the end of the fermentation periods, the seeds were dried separately in oven at 55 ^{0}C and milled into a fine flour using a disc attrition mill/ model 2002 F.H Bentall and Co.,UK) and hence milled twice in order to get fine flour. The milled samples were sieved using muslin cloth as practiced traditionally to remove the chaff and ensure uniform particle size. The sieved flours were labelled according to the fermentation period. Twenty-four hour fermentation period was coded BTF, forty-eight hours (BFE), seventy-two hours (BST) while zero hour, the unfermented portion was coded BZE. These flours were stored and later used for the chemical analysis of the nutrient and antinutrient composition.

Chemical analysis

Chemical analysis of the nutrient and antinutrient composition were determined using standard methods. Each analysis was carried out in triplicates on all samples. The micro-kjeldahl method was used to determine the crude protein while soxlet method was used to determined fat described by (17). Ash was determined using the dry ashing method as described by (18). Carbohydrate values were determined by difference method. Determination of minerals and vitamins were carried out using the method described by (19). For the antinutrient composition, Hydrogen cyanide (HCN) was quantified using (19), The estimation Phytin –Phosphate (Phytin –P or phytate) was also determined using the colorimetric procedure of described in (17). Tannin and oxalate were determined following the process described by (19).

Data analysis: The mean, standard error of the mean (SEM) and analysis of variance (ANOVA) of the data obtained from the study was computed using computer application software package, statistical package for social sciences (SPSS) version 17. Means were separated by Duncan's Multiple Range Test and significance judged at $P > 0.05$.

Results

Table 1 shows the results of the proximate composition of bambara groundnut flours

The moisture content of the flours ranges from 4.35% to 4.80% (BST and BTF). Moisture content of BZE (control) and BFE had similar values (4.55 and 4.40%). The protein content of the flours varied. The protein value of BFE was the highest (21.82%) but not significantly different from BTF (21.44%) (P>0.05) while the control and BST had the least but comparable values (20.59 and 20.39%) respectively. The fat content of the control was the highest (1.63%) and was significantly higher (P<0.05) followed by BTF and BFE (1.31 and 1.08%) while the fat content of BST was the lowest (1.02%). The ash content of BFE (2.55%) had the highest value (P<0.05) and was followed closely by BTF and BST (2.35 and 2.10%) while that of control was the lowest (2.10%). The fibre content of BZE (2.00%) was significantly higher (P<0.05). This was followed closely by BTF and BFE (1.88 and 1.86%) respectively. Meanwhile, BST had the least fibre value (1.20%). The carbohydrate content of the control was the highest (69.64%) but was not significantly higher (P>0.05) when compared with BST, BTF and BFE (68.45, 68.23 and 68.20%) respectively.

Table 2 shows the mineral composition of bambara groundnut flours from different fermentation periods respectively. For the minerals assessed, BFE had the highest values in calcium and iron (3.26 and 1.65 mg/100) (P<0.05) respectively. This was followed closely by BTF (2.67 and 1.53 mg/100) for calcium and iron content respectively. For the other minerals, BTF was significantly higher (P<0.05) in phosphorus, magnesium, manganese, copper and zinc (0.06, 3.10, 0.20, 0.21 and 0.47 mg/100) respectively.

Table 1: Proximate composition of Bambara groundnut flours at different fermentation periods (g/100).

Samples	Moisture	Protein	Fat	Ash	Fibre	Carbohydrate	Energy (KJ)))
BZE	$4.55^b \pm 0.01$	$20.59^b \pm 1.00$	$1.63^a \pm 0.01$	$1.60^c \pm 0.05$	$2.00^a \pm 0.01$	$69.64^a \pm 1.04$	1571.41
BTF	$4.80^a \pm 0.01$	$21.44^a \pm 0.38$	$1.31^a \pm 0.01$	$2.35^b \pm 0.01$	$1.88^b \pm 0.01$	$68.23^a \pm 0.43$	1550.03
BFE	$4.40^b \pm 0.01$	$21.82^a \pm 0.01$	$1.08^a \pm 0.01$	$2.55^a \pm 0.15$	$1.86^b \pm 0.00$	$68.20^a \pm 0.16$	1547.25
BST	$4.35^b \pm 0.07$	$20.39^b \pm 0.62$	$1.02^a \pm 0.00$	$2.10^b \pm 0.16$	$1.20^c \pm 0.01$	$68.45^a \pm 0.46$	1525.24

Mean ± SEM of three determinations; a-c values in the same column with different superscript letters differed from each other (P<0.05)

BZE- Zero hour unfermented bambara groundnut; BTF- Twenty-four hour fermented hour Bambara groundnut, BFE- forty-eight hour fermented bambara groundnut; BST- Seventy-two hour fermented bambara groundnut.

Table 2: Mineral composition of bambara groundnut flours at different fermentation periods (mg/100g).

Samples	Calcium	Iron	Phosphorus	Magnesium	Manganese	Copper	Zinc
BZE	$2.10^d \pm 0.01$	$1.34^c \pm 0.02$	$0.04^b \pm 0.01$	$2.90^b \pm 0.03$	$0.17^b \pm 0.01$	$018^b \pm 0.01$	$0.41^b \pm 0.00$
BTF	$2.67^b \pm 0.01$	$1.53^b \pm 0.01$	$0.06^a \pm 0.01$	$3.10^a \pm 0.05$	$0.20^a \pm 0.01$	$0.21^a \pm 0.01$	$0.47^a \pm 0.01$
BFE	$3.26^a \pm 0.00$	$1.65^a \pm 0.05$	$0.03^b \pm 0.03$	$2.68^c \pm 0.01$	$0.11^c \pm 0.01$	$0.18^b \pm 0.01$	$0.43^a \pm 0.01$
BST	$2.22^c \pm 0.02$	$1.15^d \pm 0.01$	$0.03^b \pm 0.01$	$2.45^d \pm 0.00$	$0.10^c \pm 0.00$	$0.17^b \pm 0.01$	$0.41^b \pm 0.00$

Mean ± SEM of three determinations; a-c values in the same column with different superscript letters differed from each other (P<0.05)

BZE- Zero hour unfermented bambara groundnut; BTF- Twenty-four hour fermented hour Bambara groundnut, BFE- forty-eight hour fermented bambara groundnut; BST- Seventy-two hour fermented bambara groundnut.

Table 3: Vitamin content of bambara groundnut flours at different fermentation periods.

Sample	Vit.A(I.U)	Vit.C(mg/100)	Thiamine(mg/100)	Riboflavin (mg/100)
BZE*	$3.43^{b} \pm 0.02$	$1.03^{a} \pm 0.03$	$0.88^{a} \pm 0.01$	$0.35^{a} \pm 0.00$
BTF	$3.52^{b} \pm 0.01$	$1.00^{a} \pm 0.01$	$0.84^{a} \pm 0.02$	$0.33^{a} \pm 0.00$
BFE	$4.15^{a} \pm 0.01$	$1.00^{a} \pm 0.01$	$0.85^{a} \pm 0.00$	$0.33^{a} \pm 0.00$
BST	$2.19^{c} \pm 0.01$	$1.00^{a} \pm 0.01$	$0.83^{a} \pm 0.01$	$0.33^{a} \pm 0.00$

Mean ± SEM of three determinations; a-c values in the same column with different superscript letters differed from each other (P<0.05)
BZE- Zero hour unfermented bambara groundnut; BTF- Twenty-four hour fermented hour Bambara groundnut, BFE- forty-eight hour fermented bambara groundnut; BST- Seventy-two hour fermented bambara groundnut.

Table 4: Antinutrient content of bambara groundnut flours at different fermentation periods (mg/100).

Samples	Hydrogen cyanide	Phytate	Tannin	Hemaglutinin	Oxalate
BZE*	$0.08^{a} \pm 0.00$	$4.48^{a} \pm 0.01$	$2.96^{a} \pm 0.02$	$8.54^{a} \pm 0.01$	$8.00^{a} \pm 0.10$
BTF	$0.06^{b} \pm 0.00$	$2.33^{d} \pm 0.00$	$2.66^{b} \pm 0.01$	$6.01^{d} \pm 0.01$	$7.60^{b} \pm 0.20$
BFE	$0.05^{c} \pm 0.00$	$3.89^{c} \pm 0.01$	$2.15^{c} \pm 0.01$	$6.47^{c} \pm 0.00$	$7.80^{ab} \pm 0.10$
BST	$0.06^{b} \pm 0.00$	$4.09^{b} \pm 0.01$	$2.67^{b} \pm 0.03$	$7.38^{b} \pm 0.00$	$7.80^{ab} \pm 0.10$

Mean ± SEM of three determinations; a-c values in the same column with different superscript letters differed from each other (P<0.05)
BZE- Zero hour unfermented bambara groundnut; BTF- Twenty-four hour fermented hour Bambara groundnut, BFE- forty-eight hour fermented bambara groundnut; BST- Seventy-two hour fermented bambara groundnut.

Table 3 shows vitamin composition of bambara groundnut flours from different fermentation periods. The vitamin A values of the flours varied. The vitamin A value of BFE (4.15 i.u) was significantly higher (P<0.05) while BTF and the control had comparable values (3.52 and 3.43 i.u) respectively. BST had the least value (2.1 i.u). There were no significant differences in the vitamin C values of the flour samples (P>0.05) as the results showed (1.03, 1.00, 1.00 and 1.00mg/100) for BZE, BTF, BFE and BST respectively. The thiamine and riboflavin content of the flours did not show any significant difference.

Table 4 shows the antinutrient composition of bambara groundnut flours from different fermentation periods. There were significant differences in the antinutrient content of the flours (P<0.05). The HCN value of the control (0.08mg/100g) was significantly higher (P<0.05). BTF and BST had comparable values (0.06mg/100g) while BFE had the lowest value (0.05mg/100). The phytate content of the control (4.48mg/100g) was also the highest among the flours (P<0.05). This was followed by BST and BFE (4.09 and 3.89mg/100) while BTF had the least value (2.33mg/100mg). There were significant differences in the tannin content of the flours (P<0.05). The tannin content of the control (2.96mg/100) was the highest while BFE (2.15 mg/100) was the lowest meanwhile BTF and BST had comparable results (2.66 and 2.67mg/100) respectively. There were also significant differences in the hemaglutinin content of the flours (P<0.05). For hemaglutinin content, the control also had the highest value (8.54mg/100). This was followed closely by BST and BFE (7.38 and 6.47mg/100) respectively. BTF had the least result. The oxalate values varied also. The oxalate content of the control was the highest (8.00mg/100) and was significantly higher (P<0.05) while BFE and BST had comparable results (7.80mg/100) respectively. BTF had the least value (7.60mg/100).

Discussion

The moisture content of the fermented flours BTF (4.80%) respectively were slightly higher than that of the control (4.55%). However, according to (20), these moisture levels are acceptable as they were less than 10%; hence a prolonged shelf life is expected of these flour samples. Moisture content is one of the outstanding qualities and widely used parameters in the processing and testing of foods. It is an index of water activity of many foods.

The protein content of all the flours were comparable to values reported for other Bambara groundnuts (1). The higher protein level of BFE (21.82%) might partly be due to degradation of complex protein by microorganism thereby releasing peptides and amino acids as discovered by (21). However, it is also reported that fermenting microorganisms also uses amino acid which could lower the protein content and quality of some fermented food (22, 21).

The fat content of the flours was lower when compared to other reports on nutrient composition of bambara groundnuts as found in (23). However, it was observed that there was further reduction in the fat levels of the flours as the period of fermentation increased. This reduction of fats in the flour samples could be due to hydrolysis and utilization of fats as an energy source for biochemical reactions (24, 25, 26).

The high ash value of BFE could be as a result of release of minerals due to breakdown of complex molecules which bound the minerals as fermentation period increased. A study by (27) established that ash content of foods reflects their mineral and elemental composition. Based on this fact, the higher content of BFE could be indicative of its higher mineral content.

The high fibre content of BZE (the control) showed the effect of fermentation on the other flour samples since it did not pass through the process of fermentation. Meanwhile, the reduction in the fibre complexes levels of the fermented samples could be attributable to their degradation by the microorganisms as observed by (28).

The carbohydrate content of all the flours were also comparable to values reported for Bambara groundnuts by (1). The high carbohydrate content of the control is an indication of the effect fermentation had on the other flour samples. It could also be attributable to the usage of carbohydrate which is normally preferred by lactic acid fermenters for energy production; hence the reduction in the carbohydrate levels (28) as fermentation progressed. The glucose released during fermentation is a preferred substrate for microorganisms fermenting the food and could partly explain the decrease in total carbohydrate after 24 hrs of fermentation (22).

The result of calcium levels as observed in this study does not agree with other literature. The lower levels of calcium in these flours could be attributed to the deficiency of calcium in the soil where this crops grew. Meanwhile, the observed increase in calcium content of BFE could be as a result of the fact that fermentation brings about the breakdown of phytic acid which is one of the antinutritional factors responsible for binding minerals thus making them not readily bioavailable (29) and so enhanced the ability for extracting the mineral. The result of iron levels as observed in this study was lesser when compared with report by (23). Meanwhile, the higher iron value observed in the BFE could be as a result of the fact that fermentation brings about the breakdown of complex biomolecules phytic acid is one of the antinutritional which is responsible for binding minerals thus making them not readily bioavailable (30) and so enhanced the ability for extracting the minerals. The low iron value of BST could have been utilized by the microbes involved in fermentation. Iron is essential for formation of blood and therefore is essential in the body.

The high phosphorus value found in BTF could be due to the breakdown of phytate to release more phosphorus (29).

The higher value of the magnesium, manganese, copper and zinc content in BTF could be as a result of the breakdown of complex biomolecules during fermentation and so enhanced the ability for extracting the minerals or its bioavailability (29).

The higher value observed in BFE vitamin A content could be attributed to the release of phytochemicals as observed by (31) who discovered that fermented biofortified maize for 24 and 72 hr retained 60%–100% of provitamin A carotenoids. Meanwhile, the decrease observed in sample BST according to (32) and (33) could be due to the utilization of the phytochemicals by the microorganisms fermenting the foods thus, leading to their reduction.

Fermentation had no significant effect in the vitamin C values of the flour samples though there were non-significant losses observed after fermentation. Meanwhile, this result is comparable to that reported by (23). The minute reduction observed showed that the vitamin C content of the flour samples were lost during steeping in water since vitamin C is water soluble.

The thiamine content of the flours was higher than those reported by (23). Meanwhile, the decrease observed in the thiamine content of the samples as fermentation progressed could be attributed to leaching of the vitamins into the fermentation medium since they are water soluble vitamins. This is in line with the findings of (34) who discovered that fermentation caused decreases in thiamine and riboflavin contents of yellow maize.

The riboflavin content of the flours was higher than those reported by (26). Meanwhile, the decrease observed in the riboflavin content of the samples as fermentation progressed could be attributed to the leaching into the fermentation medium since they are water soluble vitamins. This is also in agreement with the findings by (37).

The lower hydrogen cyanide (HCN) value of BFE showed that fermentation had a reducing effect on the flour sample making it safer. According to (35), a cyanide content in the range of 10-20mg per 100g of pulse is considered safe. Many legumes, except lima bean (*Phaseolus lunatus*) contain cyanide within this limit. Even though the level of HCN in the flour samples were within the safe range, fermentation also helped to reduce it further.

The lower phytate value of BTF could be due to increased activity of phytase during fermentation that led to its reduction (36). Secondly, fermentation loosens the complex matrix that embeds minerals. Both phytase and α-amylase make the matrix loose by degrading phytate and starch, respectively (29).

The lower tannin value of BFE also showed that fermentation had a reduction effect on the flour. The increased level of tannin found in SPF and SPS during fermentation could be attributed to hydrolysis of condensed tannins such as proanthocyanidin to phenols. Tannins bind minerals and reduce their bioavailability (37).

The lower hemaglutinins value of BTF signified the effect fermentation had on the flour sample bringing about a 29.62% reduction in hemaglutinin levels. Hemaglutinins and tannins impart bitter or unacceptable taste in legumes and also prevent protein digestibility (38).

The lower oxalate value of BTF also showed that fermentation caused a reduction in the oxalate level of the flour sample. The low oxalate levels might have nutritional and health benefits. This is because as stated by (39), oxalates have a negative effect on mineral availability as they interfere with absorption of divalent minerals, particularly calcium by forming insoluble salts with them. According to (40), 80% of kidney stones were composed of calcium oxalate.

Conclusion

The protein, ash and mineral content of the fermented bambara groundnut flours were found to significantly improved after fermentation and there was reduction in antinutritional factors. Meanwhile, the loss in the water soluble vitamins that leached out during fermentation as observed in the study could be replaced through fortification.

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