

**Effect of Fermentation on Antinutritional Factors and Functional Properties of Fermented Bambara Nut Flour**

**ABSTRACT**

Bambara nut (*Voandzeia subterrenea*) is a cheap source of leguminous protein that can be a good substitute for relatively expensive animal protein to reduce malnutrition. Despite its potentials, it remains underutilized owing in part to long cooking time, presence of antinutritional factors and drudgery in dehulling. In this regard, this study determined effects of fermentation on antinutritional and functional properties of bambara nut flour.

Bambara nut and wheat flour were procured from local market in Ogbomoso while pure culture of *Rhizopus oligosporus* was obtained at the Department of Food Science and Engineering, Ladoke Akintola University of Technology. Bambara nut was fermented for 12, 24, 36, 48, 60 and 72h at 32 °C and dried in oven (55 °C/24 h). The antinutritional (tannin, oxalate, phytate, and trypsin inhibitor) and functional properties (water-absorption-capacity, solubility and swelling power) of the composite flour were determined. The data obtained were subjected to descriptive and inferential statistics and significance established at  $P=0.05$ .

Respective range of values for tannin, oxalate, phytate and trypsin inhibitor were 0.08 - 0.32, 0.72 - 1.49, 0.15 - 3.64 and 0.42 - 3.25 mg/g, respectively. Water absorption capacity, solubility and swelling power ranged from 8.67 - 11.04, 52.59 - 53.07, 9.20 - 10.16 and 9.14 - 9.16%, respectively. The fermentation process reduced the antinutritional factors and increased the protein content. .

*Keywords: fermentation, bambara nut, antinutritional properties, functional properties*

**1. INTRODUCTION**

Legumes are generally known as important cheap source of protein to poor resource people in Nigeria. In recent time, traditional foods have been enriched using soybeans as the source of protein towards alleviating the associated low protein problems. Another approach to solve the problem of low intake of protein is to develop high proteinous food from other crop sources. Agricultural research traditionally has focused on staple foods while little attention has been given to underutilized and neglected crops, particularly by scientist in developed countries. One such underutilized crop is bambara nut. Bambara nut is an indigenous African crop that has been cultivated in Africa for centuries. It is a highly nutritious which accordingly plays a crucial role in human diets [1]. **One of the main attributes of bambara nut is its tolerance of drought and poor soils and its ability to yield in conditions when bambara nut fails completely.** [2] **described** bambara nut seeds as a complete balanced diet, making it a good supplement to cereal - based diets. Bambara nut contains about 63% carbohydrates, 19% protein, and 6.5% oil and is consumed in different forms which makes it rank highly as a complete food [3].

Fermented food products have contributed to the socio-economic role and improve the protein requirements of the indigenous consumers in African countries and the developing world [4]. During fermentation, microflora may produce proteolytic enzymes which may be responsible for the increase in protein digestability and also, eliminate phytic acid which contribute to the improvement of protein digestability of fermented products.

The objective of this research is to study the antinutritional and functional properties of flour from fermented bambara nut. This study could provide some basic information which would help determine an application for bambara nut in food industries.

**MATERIALS AND METHODS**

49 Bambara nuts (*Voandzeia Subterranean (L) thouars*), used for this study was obtained from a local  
50 market in Abeokuta. The species of *Rhizopus* used was obtained from the Department of Food Science  
51 and Engineering, Ladoke Akintola University, Ogbomosho.

52

## 53 **2.1 Sample Preparation**

54

55 The project was carried out at the laboratories of the Department of Food Technology, Gateway  
56 Polytechnic, Saapade.

57

### 58 **2.1.1 Subculturing of fungal cultures**

59

60 The subculture of *R. oligosporus* were prepared by the procedure described by [5]. Five hundred  
61 millilitres of PDA were prepared by using 18g of potato dextrose agar and 500 ml of distilled water, it was  
62 homogenized and sterilized. After cooling, 15 ml of PDA was dispensed into each McCartney bottle  
63 placed in a slant form. The *R. oligosporus* were subcultured singly into each McCartney bottle containing  
64 PDA and incubated at 32 °C for 4 days. One (1 ml) of each spore's suspension were thereafter taken and  
65 transferred into a haemocytometer for spores enumeration. An appropriate volume of 2 ml of these spore  
66 suspension from *R. oligosporus* was used to inoculate the bambara nut within 0-72 h fermentation  
67 period.

68

### 69 **2.1.2 Preparation of fermented bambara nut flour**

70

71 Bambara nut were fermented using the method [5]. Bambara nut (5 kg) were cleaned and washed with  
72 tap water. It was steeped in water for 24 h and dehulled. The steeped beans were boiled in the steeped  
73 water for 15 mins, drained and spread out to dry a little at room temperature. One hundred grammes of  
74 dehulled bambara nut were poured into the perforated polythene bag and an appropriate volume of 2 ml  
75 of this spore suspension of *R. oligosporus* were carefully added and thoroughly mixed. The perforated  
76 polythene bags were tightly sealed. They were incubated at 32 °C for periods of time ranging between 0  
77 and 72 h (0, 12, 24, 36, 48, 60 and 72 h). The unfermented bambara nut (0 h fermentation) were served  
78 as the control. At regular intervals of 12 h, samples were taken out for appropriate analysis. At the end of  
79 each fermentation period, samples were taken and blanched for 20 mins and then sliced into smaller  
80 units. The slices were drained and dried in an oven maintained at 55 °C for 24 h, cooled, milled and then  
81 sieved with 180 µm sieves to fine particles. The flour were packed in a polythene bags, sealed and kept in  
82 a deep freezer until required for analyses [6].

83

## 84 **2.2 Analyses**

85

### 86 **2.2.1 Antinutritional Determination**

87

88 Phytic acid and trypsin inhibitors were determined by [7] while the Tannic and oxalic acids were  
89 determined using the procedure of [8].

90

### 91 **2.2.2 Physicochemical analysis of fermented bambara nut flour**

92

93 The swelling power and solubility were determined by the method described by [9] while water absorption  
94 capacity (WAC) was carried out as described by [10]. A Rapid Visco Analyser (RVA) was used to study  
95 the pasting characteristics of the flour samples.

96

## 97 **2.3 Statistical analysis**

98

99 The data obtained from study and sensory evaluation was subjected to descriptive and inferential  
100 statistics and significance established at  $P=0.05$ .

101

## 102 **3. RESULT AND DISCUSSION**

103  
104  
105  
106  
107  
108  
109  
110  
111  
112  
113  
114  
115  
116  
117  
118  
119  
120  
121  
122  
123  
124  
125  
126  
127  
128

### 3.1 Effect of fermentation on level of antinutritional factor of bambara nut flour

The results obtained for the parameters investigated on the antinutritional properties of bambara nut are as presented in Table 1. There is a decreasing trend in tannin content within 0-72 h fermentation period ranging from 0.32±1.00 to 0.08±1.00 mg/g. Fermenting bambara nut at 0 h however gave the highest value (0.32±1.00) while the least values (0.08±1.00) were obtained at 72 h fermentation period. Reduction in tannin due to fermentation might have been caused by the activity of polyphenol oxidase or fermented microflora on tannins [11]. The observed decrease in tannin with increase in fermentation time agrees with the report of [12]. The tannin content of fermented bambara nut flour decreases from 0.33 to 0.02mg/100 g within the period of fermentation which was lower in value compared to 0.16mg/100 g reported by [11] on dehulled bambara nut flour.

There is a decreasing trend in oxalate content within 0-72 h fermentation period ranging from 1.49 ± 1.00 to 0.72 ± 1.00 mg/g. Fermenting bambara nut at 0 h however gave the highest value while the least value was obtained at 72 h. There was no significant difference in samples (E and F) at  $P= .05$ . The observed decreasing trend agrees with the findings of [11], [13] for cocoyam flour and dehulled bambara nut flour, respectively. The values obtained for the phytate content showed a decreasing trend from 3.64±1.00 to 0.15±1.00. Significant differences were observed within 0-72 h of fermentation period at  $P=.05$ . Fermenting bambara nut at 0 h however gave the highest value while the least value was obtained at 72 h fermentation period. Processing, especially fermentation, has been reported to reduce phytic acid content of cereals, legumes and tubers as a result of the activity of the endogenous phytases from both raw ingredient and inherent microorganisms which hydrolyse phytic acid in many fermented food product preparation inositol and orthophosphate [14]. Therefore, fermenting bambara nut has higher potential to enhance its nutritional value without any adverse effect and toxicity on human health.

**Table 1. Antinutritional properties of fermented bambara nut flour**

Sample	Tannin (mg/100g)	Oxalate (mg/100g)	Phytate (mg/100g)	Trypsin inhibitor (mg/100g)
A	0.32±1.00 <sup>f</sup>	1.49±1.00 <sup>f</sup>	3.64±1.00 <sup>g</sup>	3.25±1.00 <sup>g</sup>
B	0.23±1.00 <sup>e</sup>	0.96±1.00 <sup>e</sup>	2.84±1.00 <sup>f</sup>	1.90±1.00 <sup>f</sup>
C	0.21±0.46 <sup>d</sup>	0.83±1.00 <sup>d</sup>	1.37±1.00 <sup>e</sup>	0.76±1.00 <sup>e</sup>
D	0.21±0.46 <sup>d</sup>	0.81±1.00 <sup>c</sup>	1.24±1.00 <sup>d</sup>	0.70±1.00 <sup>d</sup>
E	0.19±1.00 <sup>c</sup>	0.74±0.42 <sup>b</sup>	1.20±1.00 <sup>c</sup>	0.64±1.00 <sup>c</sup>
F	0.14±1.00 <sup>b</sup>	0.74±0.42 <sup>b</sup>	0.18±1.00 <sup>b</sup>	0.56±1.00 <sup>b</sup>
G	0.08±1.00 <sup>a</sup>	0.72±1.00 <sup>a</sup>	0.15±1.00 <sup>a</sup>	0.42±1.00 <sup>a</sup>

All values are means of triplicate determinations ± standard deviation (SD). All values with different superscripts in the same column are significantly different at  $P=0.05$ .

138  
139  
140  
141  
142  
143  
144  
145  
146  
147

#### Key

Sample A = Unfermented bambara nut  
Sample B = 12 h fermented bambara nut  
Sample C = 24 h fermented bambara nut  
Sample D = 36 h fermented bambara nut  
Sample E = 48 h fermented bambara nut  
Sample F = 60 h fermented bambara nut  
Sample G = 72 h fermented bambara nut

### 3.2 Physicochemical analysis of fermented bambara nut flour

148  
149

150 The results of physicochemical analysis of the fermented bambara nut flour are shown in Table 2. The  
 151 result of swelling power ranged from  $143.24 \pm 1.00$  -  $149.28 \pm 1.00$ . There was significant difference at  
 152  $P=0.05$  in the swelling index property of the samples except for samples C and F, as well as samples B  
 153 and E. Samples A, the unfermented sample had the highest value of  $149.28 \pm 1.00$ . The significant  
 154 increase in the values may be due to the water binding properties of the legume protein (Nelson and Cox,  
 155 2000). The value is lower than that of native and ginger modified starches with values of 8.9 and 12.9,  
 156 respectively as reported by [15].

157 The water absorption capacity of the flour ranged from  $165.0 \pm 1.00$  -  $210.0 \pm 1.00$  within 0 – 72 h  
 158 fermentation. The results for the water absorption capacity showed a significant difference ( $P=0.05$ ) in the  
 159 samples with WAC decreasing as the fermentation period increases. Water absorption capacity describes  
 160 flour – water association ability under limited water supply. Both swelling index and WAC ultimately  
 161 determine the sample consistency that is solid, semi-solid or liquid. It is an index to determine the  
 162 industrial utilization of the fermented product as an ingredient. The values obtained for solubility of the  
 163 seed flour samples ranged from  $1.54 \pm 1.00$  -  $1.92 \pm 1.00\%$  with sample F having the lowest and sample  
 164 A having the highest. Solubility reflects the extent of intermolecular cross bonding with the granule [16].  
 165 This result suggests that bambara nut flour may find application in the production of some baked  
 166 products.

167  
 168 **Table 2: Physicochemical analysis of fermented bambara nut flour**

170 Sample	170 WAC (%)	170 SOL (%)	170 SP (%)
172 A	$210 \pm 1.00^e$	$1.92 \pm 1.00^a$	$149.28 \pm 1.00^e$
173 B	$200 \pm 1.00^c$	$2.01 \pm 1.00^e$	$145.58 \pm 0.35^b$
175 C	$190 \pm 1.00^d$	$1.63 \pm 1.00^f$	$144.47 \pm 1.00^a$
176 D	$180 \pm 1.00^a$	$0.50 \pm 1.00^b$	$144.14 \pm 1.00^d$
177 E	$170 \pm 0.40^b$	$0.21 \pm 1.00^a$	$145.57 \pm 0.35^b$
178 F	$170. \pm 0.40^b$	$1.42 \pm 1.00^c$	$140.47 \pm 1.00^a$
179 G	$165.0 \pm 1.00^f$	$1.54 \pm 1.00^d$	$143.24 \pm 1.00^c$

180 All values are means of triplicate determinations  $\pm$  standard deviation (SD). All values with different superscripts in the same column are significantly different at  
 181  $P=0.05$

182 **Key**

183 Sample A = Unfermented bambara nut

184 Sample B = 12 h Fermented bambara nut

185 Sample C = 24 h Fermented bambara nut

186 Sample D = 36 h Fermented bambara nut

187 Sample E = 48 h Fermented bambara nut

188 Sample F = 60 h Fermented bambara nut

189 Sample G = 72 h Fermented bambara nut

190 WAC = Water absorption capacity, SOL = Solubility, SP = Swelling power

192 **4. CONCLUSION**

193 The use of *Rhizopus Oligosporous* in the fermentation process showed reduction in  
 194 antinutritional factors with the value of tannin (0.32 – 0.08 mg/g), oxalate (1.49 – 0.72 mg/g),  
 195 phytate (3.64 – 0.15 mg/g) and trypsin inhibitor (0.42 – 0.25 mg/g) respectively. The lowest

196 values for all the investigated antinutrients were observed at 72 h of fermentation period. The  
197 functional properties (water absorption, solubility and swelling power) of bambara nut makes the  
198 seeds an ideal raw material for successful utilization in various food products and different  
199 beverages.  
200

## 201 REFERENCE

- 202 1. Jideani V.A. and Murevanitema Y.Y. Production and characterization of milk produced from bambara  
203 groundnut (*Vigna Sub terranea*) varieties. *Journal of food processing and preservation*. 2009;1745 – 4549.
- 204 2. Ouedraogo M., Ouedraogo, J.T., Tignere, J.B., Bilma, D., Dabire, C.B., Konate, G. Characterization and  
205 evaluation of accessions of Bambara groundnut (*Vigna subterranean (L.) Verdcourt*) from Burkina Faso.  
206 *Science and Nature*. 2008 Vol. 5(2); pp. 191-197.
- 207 3. Goli, A. E. Bibliographical review. In: J. Heller, F. Begemann and J. Mushonga, (Eds.), Proceedings of the  
208 Workshop on Conservation and Improvement of Bambara Groundnut (*Vigna subterranea (L.) Verdc.*), 14–16  
209 November 1995, Harare, Zimbabwe. Institute of Plant Genetics and Crop Plant Research, Gatersleben,  
210 Department of Research & Specialist Services, Harare and International Plant Genetic Resources Institute,  
211 Rome, Italy. 1997; 40: 32 – 38.
- 212 4. Achi, O.K. The potential for upgrading traditional fermented foods through biotechnology. *Africa Journal of*  
213 *Biotechnology*. 2005;4(5):375-380.
- 214 5. Olanipekun, B.F., Otunola, E.T., Adelakun, O.E. and Oyelade, O.J. Effect of fermentation with  
215 *Rhizopus oligosporus* on some physico-chemical properties of starch extracts from soybean flour. *Food and*  
216 *Chemical Toxicology*. 2009; 47(4):1401-1405.
- 217 6. Frazier, C.M. and Westhoff, D.C. Food Microbiology Fourth Edition McGraw- Hill Companies, Inc, New  
218 York. 2005; 24: 24 – 32.
- 219 7. AOAC, (2000). Official Methods of Analysis, 17<sup>th</sup> ed. Association of Official Analytical Chemists.
- 220 8. Medoua, G.N., Mbome, I. L., Agbor- Egbe, T. and Mbofung, M.F. Antinutritional Factors Changes Occuring  
221 in Trifoliolate yam (*Dioscorea dumetorum*) Tubers after Harvest. *Food Chemistry*. 2007;102(3): 716- 720.
- 222 9. Charles, A. and Guy, L. Food Biochemistry Aspen Publishers Inc. Gaitheasburg, Maryland. 1999;39 41.
- 223 10. Owuamanam, C.I., Ogueke, C.C., Achinewhu, S.C. & Barimalaa I.S. Quality characteristics of gari as  
224 affected by preferment liquor, temperature and duration of fermentation. *American Journal of Food*  
225 *Technology* 2011;Volume 6 (5);374-384.
- 226 11. Abiodun, A.O. and Adepeju, A.B. Effect of Processing on the Chemical, Pasting and Anti-Nutritional  
227 Composition of Bambara Nut (*Vigna subterranea L. Verdc*) Flour. *Advance Journal of Food Science and*  
228 *Technology*. 2011;3(4): 224-227,ISSN: 2042.
- 229 12. Onweluzo, J. C. and Nwabgwu, C.C. Fermentation of Millet (*Pennisetum americana*) and Pigeon  
230 pea (*Cajanus cajan*) Seeds to Flour Production: Effects on Composition and Selected Functional Properties.  
231 *Pakistan Journal of Nutrition*. 2009;8(6): 737- 744.
- 232 13. Oke, M.O. and Bolarinwa, I.F. Effect of fermentation on physicochemical and oxalate content of cocoyam.  
233 (*Colocasia esculenta*) Flour. *ISRN Agronomy*. 2012;Article ID 978709, Pp. 1- 4.
- 234 14. Sandberg, A. S. and Andlid, T. Phytogenic and Microbial Phytases in Human Nutrition. *International*  
235 *Journal of Food Science and Technology*. 2002;37:823-833.
- 236 15. Daramola, B. and Osanyinlusi, S.A. Investigation on modification of cassava starch using active  
237 component of ginger root (*Zingiber officinale* Roscoe). *African Journal of Biotechnology*. 2006; 5(10),  
238 Pp 917-920.
- 239 16. Amornrat, M. and Karmontip, S. “Physiochemical properties of flour and starch from jackfruit seed  
240 (*Artocarpus heterophyllus lam*) compared with modified starches”. *International journal of Food Science*  
241 *and Technology*. 2004;39, 271-276.