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Possible Effects of Fungal Fermentation on Bambara Groundnut (*Vigna Subterranea* (L) Verdc.) As a Feedstuff Resource

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Abstract: The possibility of using fungal-fermented bambara groundnut *(Vigna subterranea* (L) Verdc.) cotyledon and hulls as feedstuffs resources was investigated.The whole seed and hulls were separately fermented with a mixed culture of *Aspergillus niger*, *Aspergillus flavus*, Penicillium sp., Trichoderma sp. and yeast. Also the organisms were each used separately to ferment the seed cotyledons. The samples were then examined for proximate nutrient composition, minerals, cellulose, lignin and sugar contents as well as some anti-nutritional factors (phytates and tannins). Analysis was also carried out on the unfermented whole seed, unfermented dehulled seed, untreated hulls and naturally fermented seeds.The results indicate that the organisms have variable effects on the nutritional and anti-nutritional contents of the seeds. Fermenting the seeds with yeast or Penicillium separately significantly ($p<0.05$) increased the protein content, but a combined organism fermentation did not produce any noticeable nutritional advantage. Fermenting the seed hulls positions them for use as an excellent livestock or fish feed source.

Key words: Bambara groundnut, seeds and hulls, fungal fermentation, feedstuff

INTRODUCTION

The bambara groundnut (*Vigna subterranea* (L.) Verdc.) is an indigenous African crop that has been cultivated for centuries from Senegal to Kenya and from the Sahara to South Africa and Madagascar. It originated in the Sahelian region of present day West Africa, with its name originating from the Bambara tribe who now live mainly in Mali. Bambara groundnut is presently relegated to the underutilised status, even though it is so important in the daily diet of populations in the semi-arid regions of Africa[1,2].

One of the main attributes of bambara groundnut is its tolerance of drought and poor soils and its ability to yield in conditions when groundnut fails completely[3] . It is also relatively pest and disease-resistant. The extremely tough seed coat of the harvested seed confers resistance to weevil attack and allows the seed to be stored for long periods without loss[4]. However, despite its drought tolerant properties, bambara groundnut has largely been ignored by the Scientific Community, being regarded as a poor man's crop. The crop is cultivated for its edible seeds, which provide the major supply of dietary protein and calories in areas where it is consumed.

Bambara groundnut contains (per 100 g) 18 g protein, 6 g fat, 60 g carbohydrate, 1.5 MJ energy, 65 mg Ca, 6 mg Fe, 0.3 mg vitaminB_1, 0.1 mg vitamin B_2 and 2 mg nicotinic acid[5]. The mature dry seed contains 7.3% moisture [6]and may be eaten directly or stored as a dried pulse to be soaked and boiled before consumption[4]. Bambara groundnut may be eaten alone or mixed with sauce, condiments, spices and other foods. Bambara groundnut as a legume has been severally employed in animal feed formulations[7], in the recent time, because of the rising costs of groundnuts (peanut) cake demanded in human, livestock and fish nutrition[8].

Legumes are usually rich in anti-nutritional factors associated with fibre that reduce mineral bioavailability[9]. This has been the hindrance to their effective utilisation. According to Solomons and Ruz [10], the absorption of plant iron is usually only 1-10%, while 20-40% of zinc is absorbable. Phytates are known to reduce the bioavailability of divalent cations and these chelating agents are found in high proportions in bambara groundnut[6]. Tannins are known to form complexes with proteins, thereby making them unavailable[11] and polyphenols are common components of legumes.

However, most of the underutilised leguminous crop

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seeds in Africa are consumed via fermentation and the processing steps involved in fermentation, such as soaking and microbial action, are known to reduce the content of these anti-nutritional factors[12,13]. For example, the (Phy: Fe) and (Phy:Zn) molar ratios decreased slightly during soaking[14]. However, the fermantation of legume seeds for animal diets has been confined to bacterial action only[15].There is paucity of information on the fungal fermentation of unconventional legume seeds for feedstuffs. The present study, therefore, examines the effects of fungal fermentation on bambara groundnut as a feedstuff ingredient.

MATERIALS AND METHODS

Materials collection: The bambara groundnuts used in the study were purchased from a local market at Nri in Anmabra State, Nigeria. The seeds were screened for defective ones and transported to the laboratory in polyethylene containers. All the reagents and chemicals used in the study were of analytical grade.

Fermentation procedure: A 200 g sample each of ground whole seed and hulls was weighed separately into a plastic container with stopper. 500 mL of water was added and allowed to soak. 200 mL of nutrient solution to enhance the growth of micro-organisms [$(NH_4)_2S0_4$ 9.0 g; Urea 2.7 g and $KH_2\ PO_4$ 5.0 g in 200 mL of water] was added. The various organisms isolated were added to the broth, shaken and allowed to ferment for 72 h. The natural fermentation was done without the introduction of any organism, while the combined organism fermentation consisted of all the organisms together.

Determination of proximate and mineral compositions: Crude protein (N x 6.25), ether extract, crude fibre, ash and moisture contents of the raw and processed samples were determined according to standard methods of Association of Official Analytical Chemists[16]. Carbohydrates were obtained by the difference method. Na and K contents were determined using flame photometry (Jenway Ltd., Dunmow, Essex, UK) and other elements(Ca, Mg. Fe, Zn, Cu and Co) were determined, after wet digestion of sample ash with a mixture of nitric, sulphuric and hydrochloric acids, using atomic absorption spectrophotometer (Buck Scientific, East Norwalk, CT, USA). All determinations were carried out in triplicates.

Analysis of anti-nutritional factors: For the quantification of phytate, 8 g of finely ground sample was soaked in 200 mL of 2% HCl for 3 h and then filtered using Whatman No 1 filter paper. 50 mL of the filtrate was pipetted into 400 mL beaker and 10 mL of 0.3% ammonium thiocyanate solution was added as an indicator. 107 mL of distilled water was added to give pH 4.5. The solution was then titrated with standard ferric chloride solution containing 0.00195 g Fe mL until a brownish yellow colour persisted for 5min. The Fe equivalent was multiplied by 1.19 to get phytate-phosphorus. This was converted to phytate by multiplying the value of phytate-phosphorus by 3.55[17].

For the determination of tannin, 200 mg of finely ground sample in 10 mL of 70% acetone was extracted for 2 h at 30 °C in water-bath using Gallenkamp orbital shaker at 120 rev min^{-1}. The sample was cooled to 4°C and centrifuged for 20 min at about 3,000xg. Total polyphenols (as tannic acid equivalent) was determined in 0.05 mL aliquol in test tubes by the addition of distilled water to make it to 1.0 mL, followed by the addition of 0.5 mL of the Folin Ciocalteau reagent (freshly prepared) and 2.5 mL of sodium carbonate solution. The tubes were vortexed and the absorbance recorded at 725 nm after 40 min. The amount of total polyphenols (as tannic acid) was calculated from the standard curve. All the determinations were carried out in triplicates.

Determination of sugar , cellulose and lignin: The analysis of sugar and cellulose in the samples followed the standard procedures of AOAC[16]. The reducing sugars were determined using anthrone reagent and calculated as %glucose. For the determination of lignin, 2 mL of 72% w/w H_2S0_4 was added to 200 mg of ground sample in a 100 mL centrifuge tube and the content mixed thoroughly with a glass rod. The tube and contents were then incubated in a water bath at 30°C for 1 h, after which 50 mL of distilled water was added. This was autoclaved at 121°C, 15psi for 1h. The lignin was then filtered off with a glass fibre filter. The content was rinsed carefully into a crucible and then dried at 105°C in an oven overnight. The lignin was calculated gravimetrically as expressed in percentage.

Statistical analysis: Data were subjected to one way analysis of variance. Duncan Multiple Range test was employed to separate the differences among the means of the fermented and unfermented seeds and hulls[18].

RESULTS AND DISCUSSION

Table 1 shows the levels of phytates and tannins in the raw and fermented seeds. Tannins bind to proteins making them unavailable to the body, while phytates chelate certain divalent minerals, especially

Table 1: Some anti-nutritional content of the Bambara seed

Sample description	Tannin (ug/100g)	Phytate (mg/kg)
Unfermented Whole Sample (UWS)	322.5±0.09	338.4±0.50
Unfermented Dehulled Sample (UDS)	231.2±0.65	208.7±0.61
Naturally Fermented Sample (NFS)	12.5±0.22	253.8±0.21
Aspergillus Niger Fermented (ANF)	350.1±0.13	197.4±0.22
Aspergillus Flavus Fermented (AFF)	550.1±0.66	41.1±0.10
Trichoderma sp. Fermented (TSF)	200.1±0.16	225.6±1.02
Yeast sp. Fermented (YSF)	17.5±1.00	225.1±0.64
Penicillium sp. Fermented (PSF)	22.5±0.80	282.1±0.52
Combined Organisms Fermented (COF)	300.0±0.10	141.0±0.43
Unfermented Husk (UH)	1000.3±1.52	197.4±0.43
Combined Organisms Fermented Husk (COFH)	350.1±0.80	197.4±0.41

Table 2: Proximate composition of the Bambara seed (%)

Sample code	Moisture	Crude fibre	Fat	Protein	Ash	Carbohydrate
UWS	9.5±0.20	6.60±0.20	12.4±0.30	24.8±0.30	3.25±0.10	43.4±0.60
UDS	7.59±0.40	5.93±0.42	15.9±0.10	35.5±0.30	3.27±0.20	31.8±0.50
NFS	5.67±0.10	7.98±0.22	127±0.40	19.0±0.10	1.44±0.10	54.2±1.01
ANF	5.66±0.10	5.78±0.10	18.7±0.50	15.4±0.20	0.25±0.10	47.6±0.90
AFF	5.92±0.10	5.26±0.10	19.2±0.50	21.5±0.40	0.62±0.10	47.5±0.30
TSF	5.63±0.30	7.58±0.20	18.8±0.20	20.4±0.30	0.71±0.10	46.8±0.50
YSF	5.81±0.20	5.25±0.10	17.9±0.30	29.3±0.10	0.82±0.12	41.0±0.80
PSF	3.73±0.10	5.74±0.20	13.5±0.10	30.0±0.20	1.03±0.21	46.0±0.80
COF	5.95±0.30	4.46±0.21	17.3±0.30	24.5±0.10	1.23±0.10	46.6±1.00
UH	4.40±0.30	34.6±1.20	3.20±0.10	10.2±0.30	1.94±0.20	46.0±0.80
COFH	5.57±0.32	37.8±1.00	6.51±0.20	19.0±0.10	0.78±0.10	30.4±1.00

Zn and Fe[11,19] The present results show that both *Aspergillus* and *Trichoderma* sp. although lowering phytate level in the seed, gave unusually high tannin content, making them unsuitable, since the major reason for incorporating bambara groundnut into feedstuffs is the protein contents and bioavailability. Phytates do not impare animal performance as do tannins. For example, Onwuka[20] observed that browse phytates and oxalates did not quite impair the performance of ruminant animals significantly. Combined organisms fermentation gave an unusually high tannin content, which could be seen as the negative effect of *Aspergillus* and *Trichoderma* fermentation.

The proximate composition of raw and fermented bambara groundnut whole seeds, cotyledons and hulls is presented in Table 2 . All the organisms used appeared to have variable effects on the bambara groundnut seeds. Only Penicillium and yeast fermentation raised the protein content of the whole seed. Combining all the organisms in the fermentation did not significantly ($p>0.05$) improve upon the protein content of the cotyledons, but raised the protein, fibre and oil contents of the hulls. The results show that the only profitable organisms are Penicillium and yeast. Amadi *et al.*[21] reported an increase in protein content when bambara groundnut was fermented with Rhizopus

Table 3:Mineral contents of the Bambara seed (ppm)

Sample code	contents (ppm) Fe	Cu	Zn	Mg	Ca	Na	K	Co
UWS	22.2	ND	57.5	245.0	149	195	135	ND
UDS	20.5	ND	72.7	214.0	149	168	111	ND
NFS	58.1	ND	223.8	413.0	325	576	323	ND
ANF	29.3	ND	110.9	108.6	266	347	210	ND
AFF	34.5	ND	130.4	165.5	311	247	395	ND
TSF	55.3	ND	204.9	149.3	336	340	247	ND
YSF	73.2	ND	166.9	65.4	500	517	426	ND
PSF	50.3	ND	198.2	90.2	281	391	287	ND
COF	45.2	ND	206.9	154.0	379	961	413	ND
UH	68.7	ND	297.1	374.0	484	697	469	ND
COFH	66.8	ND	223.5	128.0	414	517	362	ND

ND = Not detected.

Table 4: Cellulose, lignin and sugar content of the Bambara seed

Sample code	percent (%) Cellulose	Lignin	Sugar U.W.S
	11.2	9.5.0	0.07
UDS	8.20	8.50	0.07
NFS	22.0	20.5	0.12
ANF	18.1	6.60	0.46
AFF	26.8	19.9	0.43
TSF	11.6	18.1	0.46
YSF	16.1	28.2	0.46
PSF	12.9	16.0	0.43
COF	16.8	11.7	0.46
UH	36.2	18.1	0.10
COFH	54.4	17.7	0.44

oligosporus and *Rhizopus stolonifer*. Also, it could be said that dehulling brought about significant ($p<0.05$) increases in protein and fat contents.

Table 3 shows the mineral concentrations in the raw and fermented bambara groundnut seeds and hulls. Overall, fungal fermentation improved upon the mineral composition.

Dehulling raised the Zn content, but significantly lowered the Na and K contents. Anyika[22] also observed significant increase in mineral balance in animals fed dehulled bambara groundnuts compared to whole seeds. Natural fermentation generally raised the mineral contents and as it is known that bacteria play prominent roles in the traditional fermentation, single fungal fermentation may not give the needed mineral boost. However, combined fungal fermentation also significantly ($p<0.05$) raised the mineral content.

The changes in cellulose, lignin and sugar contents with respect to fungal fermentation of bambara groundnut seeds and hulls are presented in Table 4. Both natural fermentation and fungal fermentation separately and in combination increased the cellulose, lignin and sugar contents in the bambara groundnut. All animals which are adapted to utilization of cellulose, especially small ruminants and herbivorous fishes would be benefited by fungal fermentation, if bambara groundnut is to be incorporated into the feed. The generally low reducing

sugar content may not pose a problem to many species, as their digestive systems can easily break down oligo-and polysaccharides to simple sugars.

CONCLUSIONS

This study has shown that fungal fermentation of bambara groundnut, especially *Penicillium* sp. and yeast, improved upon the nutrient potentials of the seed as a feedstuff resource. *Aspergillus* sp. and *Trichoderma* sp. may not be useful in the fermentation.

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