

Effect of *Aspergillus niger* and *Trichoderma viride* on the nutritional composition of alkali pretreated groundnut husk.

ABSTRACT

The effects of *Aspergillus niger* and *Trichoderma viride* on the nutritional composition of 2M NaOH pre-treated groundnut husk (GH) were studied. The pre-treated groundnut husk (GH) was subjected to monoculture fermentation using *A. niger* and *T. viride*. The lignin and cellulose contents, proximate nutrient composition, anti-nutrients, minerals and amino acid contents were evaluated. The proximate composition of GH differed from one monoculture treatment to another. *T. viride* treatment gave the best as a result of 73% increase in protein with significant ($p<0.05$) decrease in anti-nutritional content such as phytate (51.22g/100g) and alkaloid (7g/100g). Significantly ($p<0.05$) higher values of phosphorous (774 ppm), zinc (20.42 ppm) and magnesium (6.94 ppm) were observed for all fermented GH compared to untreated GH (control), while significantly ($p<0.05$) lower values of copper (0.02 ppm), sodium (30 ppm) and iron (0.11 ppm) were observed for all fermented GH compared to control. Except for aspartate, there was general decrease in amino acid content of all cultured with *T. viride* and *A. niger* GH compared to the standard feed protein used to standardize the amino acid analyser. The amino acid contents of cGH *A. niger* were slightly higher than cGH *T. viride*. The results of this study indicate that *T. viride* is a potentially viable microorganism for the production of enriched and safe GH as an animal feed alternative.

Keywords: *Trichoderma viride*, Solid State Fermentation (SSF), Groundnut Husk, *Aspergillus niger*, Anti-Nutrients

1. INTRODUCTION

The development of new technologies in agriculture has resulted in an increase in the production of many types of foods. It has given rise to wastes in large quantities thus leading to environmental pollution associated with several health hazards. The waste material from food processing industries contains some useful organic substances like polysaccharides, sugars, amino acids, starch, pectic substances and other compounds of nutritional significance including fibers, vitamins and minerals [1]. Among various forms of biomass, agricultural crop residues

are particularly well suited for energy applications because of its large-scale availability, low cost and environmentally benign production [2]. Many microorganisms including fungi and bacteria had been found to degrade cellulose and other plant cell wall fibers. In nature, degradation of cellulosic biomass is performed by mixtures of hydrolytic enzymes collectively known as cellulases, however filamentous fungi are preferred for commercially important enzymes production, because the level of the enzymes produced by these cultures is higher than those obtained from yeast and bacteria [3]. *Aspergillus* and *Trichoderma* are the most important and safe microorganisms for industrial use and are more potent producers of cellulase [4]. The enzymatic degradation of waste cellulose by fungal cellulases has been suggested as a feasible alternate for the conversion of lignocellulosics into fermentable sugars and fuel ethanol [5]. *Aspergillus niger* is a fungus and one of the most common species of the genus *Aspergillus*. Various strains of *A. niger* are used in the industrial preparation of citric acid (E330) and gluconic acid (E574) and have been assessed as acceptable for daily intake by the World Health Organisation. *A. niger* fermentation is "generally recognized as safe" (GRAS) by the United States Food and Drug Administration under the Federal Food, Drug and Cosmetic Act [6]. *Trichoderma* has been found to be one of the most effective for hydrolysis of cellulosic materials which produces an extracellular, stable, and efficient cellulase enzyme system. *Trichoderma* is a partially catabolite repressed, hypercellulolytic mutant strain, widely studied, with improved enzyme production capabilities, when compared to the wild-type and some other strains [7]. Lignocellulosic materials such as crop residues, grasses, wood chips, sawdust and animal waste (solid) can be considered as a potential source for large amount of low-cost utilizable products. Lignin is a hard substance present in lignocelluloses which leads to a protective barrier that prevents plant cell destruction by fungi and bacteria for conversion. Cellulose, a widely distributed long-chain polymeric and skeletal polysaccharide of β -glucose, is the most abundant renewable natural product obtained in the biosphere mostly in many farm residues and its potential as an alternative energy source has stimulated researches on converting cellulose to soluble sugars [8, 9]. The ability of cellulolytic microorganisms to degrade cellulose vary greatly with the physico-chemical characteristics of the substrate, such as, the size and permeability of cellulolytic enzymes. Cellulase enzymes are complex modular proteins that are comprised of one or more catalytic domains and substrate binding domains, which hydrolyze cellulose to β -glucose. Pretreatment methods change the physical and chemical structure of lignocellulosic

biomass and improve hydrolysis rates [10, 11]. Groundnut also known as peanut (*Arachis hypogaea*), is a species in the legume or "bean" family (Fabaceae). Groundnut shell (GS), obtained after the removal of nuts from groundnut pod is an agricultural by-product (termed 'Husk' after milling) and a valuable resource that can be a potential feedstock for production of fermentable sugars for animal feeding after processing due to a high amount of lignin, cellulose, fiber, protein, carbohydrate. This residue is often thrown away, burnt, or ploughed into the soil to improve its nitrogen content, hence the objective to pretreat the sample and quantify the proximate content, anti-nutritional content, mineral composition and amino acids profile to evaluate its potential to form the principal feed in small scale animal farming systems during dry seasons.

2. MATERIALS AND METHODS

2.1 Materials

Groundnut husk (GH) was obtained from a local farmer in Zaria. Potato Dextrose, 5g of powdered glucose were purchased from a chemical retail shop. Hydrochloric acid, nitric acid, sodium hydroxide, sodium citrate, citric acid, magnesium sulphate, zinc chloride, potassium nitrate, iron chloride, calcium chloride used in this study were all of analytical grade.

2.2 Microorganisms

Aspergillus niger was isolated from groundnut seed and *T. viride* was isolated from cultivated soil. The sources were suspended in 10 mL distilled water after which it was serially diluted in 9 mL distilled water. 0.5 mL of the solution was pour plated on PDA plates, incubated in Gallenkamp Incubator (model IH-150) at 28 °C for 72hr. Both organism morphology and structure as described in Fungal Atlas [12] were used to identify the organism, these were sub-cultured on other plates and slants to obtain pure strain. They were maintained on PDA medium and stored at 4 °C.

2.3 Preparation of groundnut husk suspension

Groundnut shells were collected from the open field, milled and sieved to 0.2 mm particle size. This was pretreated with 500 mL 2M sodium hydroxide for 1hour, washed with distilled water until pH is neutral, then dried in oven at 60 °C for 7hours. Ten grams (10g) was weighed into a

250 mL Erlenmeyer flask, moistened with 40 mL Basal medium [13], autoclaved at 121 °C for 15 minutes ready for use.

2.4 Inocula Preparation

The inoculum of each isolate was prepared by measuring 10 mL sterile distilled water into each agar slants and using sterile wire loop to wash the sporophore into the water. Each isolate was subsequently diluted with more sterile distilled water until a sporophore count of approximately 5×10^6 per ml was obtained using a Haemocytometer 0.25mm (New Berger) [14].

Potato Dextrose (PD) broth was prepared by dissolving 2g of glucose in 1L of distilled water used to boil 200g of peeled potatoes (sweet potatoes) for 1hr. The resulting solution was filtered through muslin cloth and made up to 1L with Basal medium consisting of the different salts (Czapek modified medium) [13]. PD broth (25 mL) was measured into 150 mL conical flasks corked with cotton wool and foil paper. These were autoclaved at 121°C for 15 minutes before use. Sporophores of *A. niger* and *T. viride* (1ml) each were inoculated into 25 mL PD broth and placed on a Gallenkamp shaker (Lab-line orbit environ-shaker 18 No. 3527-1/34) at 200rpm for 5 days [15].

2.5 Solid state fermentation of GH

Actively growing fungi (in form of flocs), 10 mL each were inoculated directly on moistened GH in a sterile environment by opening a small part of the flask (mixed fermentation). Twenty milliliter (20 mL) of each fungus was inoculated in different flasks (monoculture fermentation). Each treatment was carried out in duplicate, 10g of GH without inoculation of microorganism was set aside as the control. All flasks were incubated at 28 °C for 14days.

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2.6 Chemical Composition

Lignin and Cellulose contents were quantified as described by Irfan *et al.*, [16]. Proximate composition (moisture, ash, lipid, fiber, protein and carbohydrate) were determined by method of AOAC [17] and anti-nutrient (phytate, oxalate, alkaloids, tannin and saponin) content was

determined. Mineral constituents were assayed using atomic absorption spectroscopy and amino acid composition was assayed using Technicon sequential Multi-Sample Amino Acid Analyzer (TSM) as described by Benitez [18].

2.7 Statistical analysis

SPSS statistical package version 20 was used to analyse data. Results were expressed as mean $\pm$ standard error (SE) and data was analysed by one-way analysis of variance (ANOVA). The difference between fermentations was computed using DUNCAN range multiple test, p value less than 0.05 was considered significant ($p < 0.05$).

3.0 RESULTS AND DISCUSSION

3.1 Effect of alkaline pretreatment on lignin and cellulose content of GH

Pretreatment has a reductive effect on the lignin content of GH and cellulose content increased significantly ($p < 0.05$) with respect to control (Figure 1).

Cellulose and lignin show a negative correlation with decrease in lignin content leading to an increase in cellulose content. This might be attributed to the fact that the 2M NaOH pretreatment disrupt the compact nature of lignin which encapsulates nutrients.

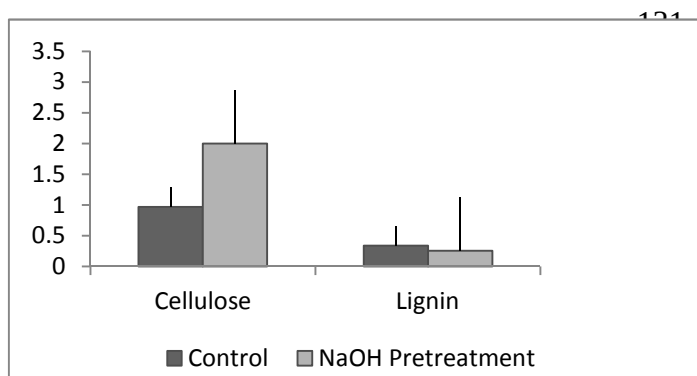


Figure 1: Effect of pretreatment on yields of lignin and cellulose content. Vertical bar shows SE.

3.2 Proximate Composition

Percentage changes in proximate composition of untreated (control), pretreated and treated (fermented) groundnut husk (GH) are shown in Table 1. The moisture and fat contents of all

140 fermented GH treatments decreased significantly ($p<0.05$) when compared with control (GH).
 141 cGH *A. niger* have the least fat value, 0.4% and chemical pretreated GH on which *T. viride*
 142 acted on (cGH *T. viride*) having the highest fat value (2.13%). The ash content for chemical
 143 pretreated GH with respect to the control (uGH) slightly decreased, with cGH *A. niger* having the
 144 least value (1.35%). No difference was observed in protein content of control (GH) and
 145 chemically pretreated GH (cGH) however the content in other fermentations were observe to
 146 increase, 10.45% and 9.37% for cGH *T. viride* and cGH *A. niger* respectively. However
 147 significant ($p<0.05$) decrease in carbohydrate was observed for cGH *T. viride*.

148 The results of proximate composition and analyzed fibre components in all treatment show an
 149 increase in the protein contents, this was partly due to the ability of the enzymes to increase the
 150 bioavailability of the protein hitherto encapsulated in the cell. Decrease in carbohydrate may be
 151 due to the ability of *A. niger* or *T. viride* to break polysaccharides into monomer sugars which
 152 are easily available for utilization by the organisms as previously reported by Iyayi and Aderolu
 153 [19].

154 Table 1: Proximate composition (%) of pretreated and fermented GH

Sample	Moisture	Fat	Ash	Protein	Fiber	Carbohydrate
GH	5.61±0.00 ^d	2.39±0.01 ^d	2.53±0.010 ^c	4.87±0.035 ^a	71.28±0.73 ^a	84.82±0.14 ^b
cGH	2.81±0.28 ^a	2.11±0.02 ^b	2.47±0.055 ^b	4.87±0.17 ^a	83.23±2.23 ^d	85.76±0.32 ^c
cGH <i>A. niger</i>	4.20±0.01 ^b	0.48±0.354 ^a	1.35±0.150 ^a	9.37±0.14 ^b	72.78±1.02 ^b	84.60±0.66 ^b
cGH <i>T. viride</i>	5.40±0.014 ^c	2.13±0.021 ^c	2.76±0.025 ^d	10.45±0.00 ^c	78.67±0.14 ^c	79.27±1.22 ^a

155 Results are presented as Mean ± SE. Values in the same column with different superscript are
 156 significantly ($p<0.05$) different. GH - Groundnut husk without treatment (control); cGH –
 157 chemical pretreated GH; cGH *A. niger* - chemical pretreated GH in which *A.niger* was
 158 inoculated; cGH *T. viride* - chemical pretreated GH in which *T. viride* was inoculated.

159 3.3 Anti-nutrients Content

160 Alkaloid, Phytate and Saponin contents of all fermented GH were observed to significantly
 161 ($p<0.05$) decrease with respect to untreated GH, with cGH *T. viride* having the least value

(0.17mg) for saponin. Oxalate content of cGH *A. niger* (22.03mg) while cGH *T. viride* (32.80mg) increased significantly ($p<0.05$). Anti-nutrient contents were observed to be relatively lower in cGH *T. viride* with respect to the control as shown in Table 2.

The improvement seen in the available minerals may be explained by the ability of the organisms to elicit phytase which increases the availability of phosphorus and this may invariably lead to improvement in the availability of other minerals such as zinc, manganese, calcium, copper and iron that are susceptible to chelation with phytate [20].

Table 2: Anti-nutrient content of pretreated and fermented groundnut husk

Sample	Saponin mg/100g	Alkaloid mg/100g	Oxalate mg/100g	Phytate g/100g	Tannin g/100g
GH	0.80±0.100 ^b	1.03±0.020 ^c	22.09±0.315 ^a	53.42±98.0 ^c	1.32±0.25 ^b
cGH	4.13±0.040 ^d	0.75±0.010 ^a	28.03±0.025 ^b	40.05±5.50 ^a	3.29±1.59 ^d
cGH <i>A. niger</i>	1.89±0.025 ^c	1.13±0.015 ^d	22.03±0.370 ^a	47.98±1.50 ^b	1.64±0.79 ^c
cGH <i>T. viride</i>	0.17±0.015 ^a	0.97±0.010 ^b	32.80±0.800 ^c	41.63±3.50 ^a	0.98±0.96 ^a

Results are presented as Mean ± SE. Values in the same column of different treatment with different superscript are significantly ($p<0.05$) different. GH - Groundnut husk without treatment (control); cGH – chemical pretreated GH; cGH *A.niger* - chemical pretreated GH in which *A.niger* was inoculated; cGH *T. viride* - chemical pretreated GH in which *T. viride* was inoculated.

3.4 Mineral Composition

Copper, Iron, Sodium and Manganese contents in all fermentations were observed to decrease significantly with Copper having the least value (0.02 ppm for cGH *T. viride* and cGH *A. niger*) followed by Iron (30 ppm for cGH *T. viride*).

Reduction of some anti-nutrients such as alkaloids, tannins and phytate in various treatments are consistent with report of Wyss *et al.*, [21] that a significant reduction of various anti-nutritional components were observed during processing.

Table 3: Mineral composition (ppm) of pretreated and fermented groundnut husk

Sample	Cu	Zn	Mn	Mg	Fe	Ca	Na	P
GH	0.12±0.	1.40±0.1	0.81±0.0	6.94±0.	5.93±0.	15.58±0.0	880±2	774±14.
(control)	00 ^b	6 ^b	0 ^c	10 ^d	02 ^d	8 ^b	0 ^b	31 ^b
cGH	0.20±0.	3.01±0.2	0.46±0.2	1.54±0.	1.89±0.	33.17±0.1	670±1	774.99±
	00 ^a	3 ^c	2 ^{bc}	06 ^b	01 ^b	3 ^c	0 ^b	14.31 ^b
cGH A.	0.02±0.	20.42±0.	0.32±0.0	5.35±0.	3.18±0.	33.73±0.1	620±3	774.99±
niger	001 ^a	19 ^d	1 ^{ab}	02 ^c	01 ^c	3 ^c	0 ^b	14.31 ^b
cGH T.	0.02±0.	0.76±0.0	0.00±0.0	0.18±0.	0.11±0.	0.794±0.2	30±0.0	301.39±
viride	00 ^a	0 ^a	0 ^a	001 ^a	02 ^a	6 ^a	^a	21.1 ^a

Results are presented as Mean ± SE. Values in the same column with different superscript are significantly (p<0.05) different. GH - Groundnut husk without treatment; cGH – chemical pretreated GH; cGH *A. niger* - chemical pretreated GH in which *A. niger* was inoculated; cGH *T. viride* - chemical pretreated GH in which *T. viride* was inoculated.

3.5 Amino acid Composition

As shown in Table 4, Aspartic acid was observed to be highest in all fermented GH samples, with cGH *A. niger* sample been the highest. Glutamic acid, proline and leucine were observed to have a higher concentration (longer peak) than other amino acids, cGH *T. viride* was observed to have glutamic acid concentration of 10.50. The highest concentration (8.58g/100g) of proline was recorded for cGH *A. niger*. Lysine, tyrosine, threonine, arginine and histidine contents were all observed to be low in concentration in all fermented GH. However, sulphur containing amino acids such as cysteine and methionine were not detected in all GH samples.

The decrease in amino acid content can be attributed to the uptake of these amino acids by the fungi for proper metabolism and growth.

Table 4: Amino acid concentration (g/100g) of pretreated and fermented GH

Amino	Standard	GH	cGH	A.	cGH	T.
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Acid		(Control)	<i>niger</i>	<i>viride</i>
Lys	13.20	4.56	5.51	4.00
His	15.28	2.41	2.32	2.38
Arg	9.10	3.15	2.98	2.98
Asp	8.68	13.60	10.41	13.00
Thr	7.81	3.29	3.21	3.01
Ser	8.08	4.47	4.35	4.17
Glu	11.40	10.87	10.30	10.52
Pro	15.95	8.82	8.58	8.24
Gly	6.16	4.76	4.71	4.53
Ala	9.97	4.47	4.23	4.39
Val	9.76	4.90	4.78	4.72
Ile	8.97	3.69	3.69	3.52
Leu	8.07	6.34	6.16	6.28
Tyr	15.93	1.32	1.16	1.16
Phe	10.59	4.05	3.96	3.78

198 GH - Groundnut husk without treatment (control); cGH – chemical pretreated GH; cGH *A. niger*
199 - chemical pretreated GH in which *A.niger* was inoculated; cGH *T. viride* - chemical pretreated
200 GH in which *T. viride* was inoculated.

201
202 The present study indicate that chemical pretreated GH inoculated with *T. viride* (cGH *T. viride*)
203 was best in terms of increase in protein content and decrease in anti-nutrients. Although the
204 mineral content is low and the amino acid content decreased with respect to the control (GH)
205 and thus are not up to standard. Hence such economic and potential processing methods could be
206 adapted for versatile utilization of groundnut husk treated with *T. viride* as protein source.
207 Incorporation of such fermented groundnut husk in non-ruminant diet will clearly reduce over
208 dependence on common cereals or legumes for meeting protein source.
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