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Effect of *Aspergillus niger* and *Trichoderma viride* on the Nutritional Composition of Alkali Pretreated Groundnut Husk

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Authors' contributions

This work was carried out in collaboration between both authors. Author HMI designed the study and wrote the protocol. Author NA managed the analyses of the study, performed the statistical analysis, wrote the first draft of the manuscript and managed literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The effects of *Aspergillus niger* and *Trichoderma viride* on the nutritional composition of 2 M 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 (51.22 g/100 g) and alkaloid (7 g/100 g). 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

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standard feed protein used to standardize the amino acid analyser. The amino acid contents of $_C$GH *A. niger* were slightly higher than $_C$GH *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, 5 g 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 (modelIH-150) at 28ºC for 72 hr. 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 4ºC.

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 2 M sodium hydroxide for 1 hour, washed with distilled water until pH is neutral, then dried in oven at 60ºC for 7 hours. Ten grams (10 g) was weighed into a 250 mL Erlenmeyer flask, moistened with 40 mL Basal medium [13], autoclaved at 121ºC 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 0.25 mm (New Berger) [14].

Potato Dextrose (PD) broth was prepared by dissolving 2 g of glucose in 1 L of distilled water used to boil 200 g of peeled potatoes (sweet potatoes) for 1 hr. The resulting solution was filtered through muslin cloth and made up to 1 L 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 121ºC for 15 minutes before use. Sporophores of *A. niger* and *T. viride* (1 ml) 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 200 rpm 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, 10 g of GH without inoculation of microorganism was set aside as the control. All flasks were incubated at 28ºC for 14 days.

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±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. 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 (Fig. 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 2 M NaOH pretreatment disrupt the compact nature of lignin which encapsulates nutrients.

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

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

3.3 Anti-nutrients Content

Alkaloid, Phytate and Saponin contents of all fermented GH were observed to significantly ($p<0.05$) decrease with respect to untreated GH, with $_C$GH *T. viride* having the least value (0.17 mg) for saponin. Oxalate content of $_C$GH *A. niger* (22.03 mg) while $_C$GH *T. viride* (32.80 mg) increased significantly ($p<0.05$). Anti-nutrient contents were observed to be relatively lower in $_C$GH *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].

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 $_C$GH *T. viride* and $_C$GH *A. niger*) followed by Iron (30 ppm for $_C$GH *T. viride*) as shown in Table 3.

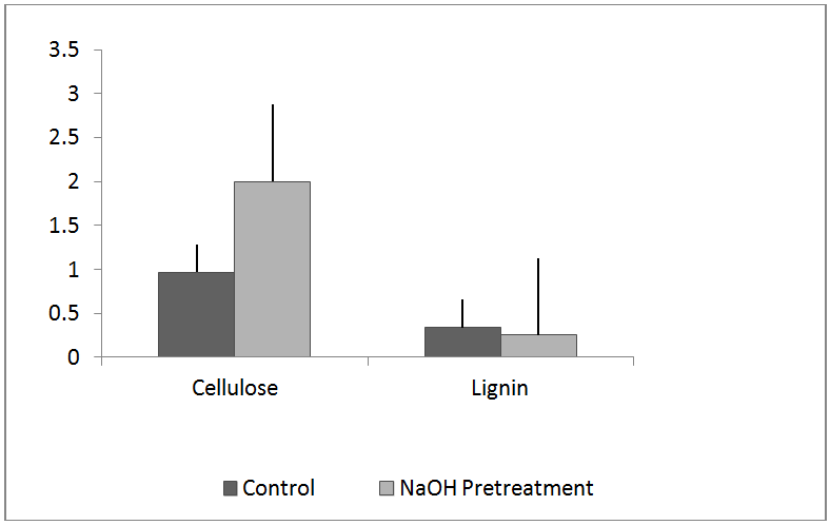

Fig. 1. Effect of pretreatment on yields of lignin and cellulose content. Vertical bar shows SE

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}
$_{C}$GH	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}
$_{C}$GH *A. niger*	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}
$_{C}$GH *T. viride*	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}

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 (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

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}
$_{C}$GH	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}
$_{C}$GH *A. niger*	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}
$_{C}$GH *T. viride*	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

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

Sample	Cu	Zn	Mn	Mg	Fe	Ca	Na	P
GH (control)	0.12±0.00^{b}	1.40±0.16^{b}	0.81±0.00^{c}	6.94±0.10^{d}	5.93±0.02^{d}	15.58±0.08^{b}	880±20^{b}	774±14.31^{b}
$_{C}$GH	0.20±0.00^{a}	3.01±0.23^{c}	0.46±0.22^{bc}	1.54±0.06^{b}	1.89±0.01^{b}	33.17±0.13^{c}	670±10^{b}	774.99±14.31^{b}
$_{C}$GH *A. niger*	0.02±0.001^{a}	20.42±0.19^{d}	0.32±0.01^{ab}	5.35±0.02^{c}	3.18±0.01^{c}	33.73±0.13^{c}	620±30^{b}	774.99±14.31^{b}
$_{C}$GH *T. viride*	0.02±0.00^{a}	0.76±0.00^{a}	0.00±0.00^{a}	0.18±0.001^{a}	0.11±0.02^{a}	0.794±0.26^{a}	30±0.0^{a}	301.39±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

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.

3.5 Amino acid Composition

As shown in Table 4, Aspartic acid was observed to be highest in all fermented GH samples, with $_{C}$GH *A. niger* sample been the highest. Glutamic acid, proline and leucine were observed to have a higher concentration (longer peak) than other amino acids, $_{C}$GH *T. viride* was observed to have glutamic acid concentration of 10.50. The highest concentration (8.58 g/100 g) of proline was recorded for $_{C}$GH *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.

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

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

Amino acid	Standard	GH (Control)	$_c$GH *A. niger*	$_c$GH *T. viride*
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

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

4. CONCLUSION

The present study indicate that chemical pretreated GH inoculated with *T. viride* ($_c$GH *T. viride*) was best in terms of increase in protein content and decrease in anti-nutrients, although the mineral content is low and the amino acid content decreased. Hence such economic and potential processing methods could be adapted for versatile utilization of groundnut husk treated with *T*. *viride* as protein source. Incorporation of such fermented groundnut husk in non-ruminant diet will clearly reduce over dependence on common cereals or legumes for meeting protein source.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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