

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 (774ppm), zinc (20.42ppm) and magnesium (6.94ppm) were observed for all fermented GH compared to untreated GH (control), while significantly ($p<0.05$) lower values of copper (0.02ppm), sodium (30ppm) and iron (0.11ppm) 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

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]. 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. Pretreatment methods change the physical and chemical structure of lignocellulosic biomass and improve hydrolysis rates. 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, ploughed into the soil to improve its nitrogen content, or used to form the principal feed in small scale animal farming systems during dry seasons.

2.0 METHODS

2.1 Microorganisms

A. niger was isolated from groundnut seed, *T. viride* was isolated from cultivated soil and both organisms were morphologically and structurally identified as described in Fungal Atlas [3]. They were maintained on PDA medium and stored at 4°C.

2.2 Preparation of Groundnut husk

Groundnut shells were collected from the open field, milled and sieved to 0.2mm particle size. This was pretreated with 500ml 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 250ml Erlenmeyer flask, moistened with 40ml Basal medium [4], autoclaved at 121°C for 15min ready for use.

2.3 Inocula Preparation

The inoculum of each isolate was prepared by measuring 10ml 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 [5].

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) [4]. PD broth (25ml) was measured into 150ml conical flasks corked with cotton wool and foil paper. These were autoclaved at 121⁰C for 15minutes before use. Sporophores of *A. niger* and *T. viride* (1ml) each were inoculated into 25ml PD broth and placed on a shaker at 200rpm for 5 days [6].

2.4 Solid State fermentation of GH

Actively growing fungi (in form of flocs), 10ml each were inoculated directly on moistened GH in a sterile environment by opening a small part of the flask (mixed fermentation). Twenty milliliter (20ml) of each fungus was inoculated in different flasks (monoculture fermentation). Each treatment was carried out in duplicate and flasks were incubated at 28⁰C for 14days.

2.5 Chemical Composition

Lignin and Cellulose contents were quantified as described by Irfan *et al.*, (2011) [7]. Proximate composition (moisture, ash, lipid, fiber, protein and carbohydrate) were determined by method of AOAC (2006) [8] 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 (1998)[9].

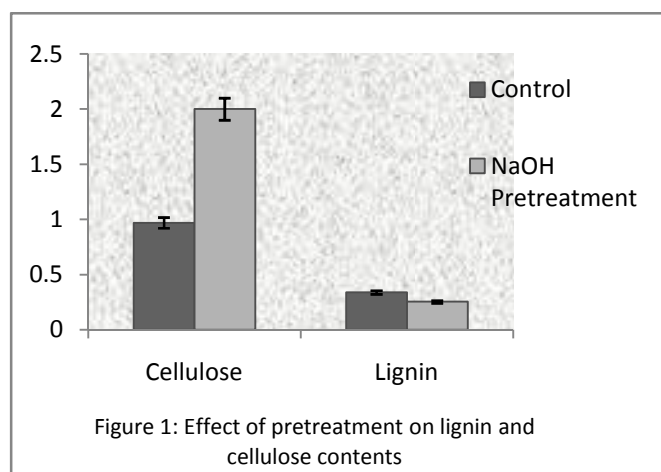
2.6 Statistical Analysis

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).

3.0 RESULT

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

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



87 3.2 Proximate Composition

88 Percentage changes in proximate composition of untreated, pretreated and treated groundnut
89 husk (GH) are shown in Table 3.1. The moisture and fat contents of all fermented GH treatments
90 decreased significantly ($p<0.05$) when compared with untreated GH. *cGH_{A. niger}* have the least
91 fat value, 0.4% and chemical pretreated GH on which *T. viride* acted on (*cGH_{T. viride}*) having the
92 highest fat value (2.13%). The ash content for chemical pretreated GH with respect to the control
93 (uGH) slightly decreased, with *cGH_{A. niger}* having the least value (1.35%). No difference was
94 observed in protein content of untreated GH (uGH) and chemically pretreated GH (*cGH*)
95 however the content in other fermentations were observe to increase, 10.45% and 9.37% for
96 *cGH_{T. viride}* and *cGH_{A. niger}* respectively. However significant ($p<0.05$) decrease in carbohydrate
97 was observed for *cGH_{T. viride}*

98 Table 3.1: Proximate Composition (%) of pretreated and fermented GH

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

99 Results are presented as Mean $\pm$ SE. Values in the same column with different superscript are
100 significantly (p<0.05) different

101 3.3 Anti-nutrients Content

102 Alkaloid, Phytate and Saponin contents of all fermented GH were observed to significantly
103 (p<0.05) decrease with respect to untreated GH, with *cGH_{T. viride}* having the least value (0.17mg)
104 for saponin. Oxalate content of *cGH_{A. niger}* (22.03mg) while *cGH_{T. viride}* (32.80mg) increased
105 significantly (p<0.05). Anti-nutrient contents were observed to be relatively lower in *cGH_{T. viride}*
106 with respect to the control as shown in Table 3.3.

107 Table 3.3: Anti-nutrient Content of untreated, pretreated and fermented groundnut husk

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

108 Results are presented as Mean $\pm$ SE. Values in the same column of different treatment with
109 different superscript are significantly (p<0.05) different

110 3.4 Mineral Composition

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

114 Table 3.4: Mineral Composition (ppm) of untreated, pretreated and fermented groundnut husk

Sample	Cu	Zn	Mn	Mg	Fe	Ca	Na	P
GH	0.12 $\pm$ 0.00 ^b	1.40 $\pm$ 0.16 ^b	0.81 $\pm$ 0.00 ^c	6.94 $\pm$ 0.10 ^d	5.93 $\pm$ 0.02 ^d	15.58 $\pm$ 0.08 ^b	880 $\pm$ 20 ^b	774 $\pm$ 14.31 ^b

cGH	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
cGH_A	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
niger								
cGH_T	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
viride								

Results are presented as Mean ± SE. Values in the same column with different superscript are significantly (p<0.05) different

3.5 Amino acid Composition

As shown in Table 3.5, 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.

Table 3.5 Amino acid concentration (g/100g) of untreated and fermented GH

Amino Acid	Standard	GH (Control)	cGH_A.niger	cGH_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

126 DISCUSSION

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

130 The results of proximate composition and analyzed fibre components in all treatment show an
131 increase in the protein contents, this was partly due to the ability of the enzymes to increase the
132 bioavailability of the protein hitherto encapsulated in the cell. Decrease in carbohydrate may be
133 due to the ability of *A. niger* or *T. viride* to break polysaccharides into monomer sugars which
134 are easily available for utilization by the organisms as previously reported by Iyayi and Aderolu
135 (2004) [9]. The improvement seen in the available minerals may be explained by the ability of
136 the organisms to elicit phytase which increases the availability of phosphorus and this may
137 invariably lead to improvement in the availability of other minerals such as zinc, manganese,
138 calcium, copper and iron that are susceptible to chelation with phytate [10].

139 Reduction of some anti-nutrients such as alkaloids, tannins and phytate in various treatments are
140 consistent with report of Wyss *et al.*, [11] that a significant reduction of various anti-nutritional
141 components were observed during processing.

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

144 CONCLUSION

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

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