
Biodegradation of Groundnut Shells by *Aspergillus niger* and *Trichoderma viride* for Feed Potential Enhancement

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Abstract

High cost of livestock feed ingredients in Nigeria has necessitated the upgrading of agricultural wastes for use as feed materials. The abilities of *Aspergillus niger* and *Trichoderma viride* to enhance the feed potential of groundnut shells through biodegradation was therefore investigated. Proximate, anti-nutrient, and energy contents were used as indices. Pulverised groundnut shells were biodegraded with *A. niger* and *T. viride* for seven days at $25\pm 2^{\circ}\text{C}$. Proximate, anti-nutrient, and energy compositions of the degraded and non-degraded shells were determined and the values obtained compared. The crude protein contents of *A. niger*-degraded groundnut shells (ANDGS) (7.52%) and *T. viride*-degraded groundnut shells (ANDGS) (7.60%) were significantly higher ($p < .05$) than that of the raw shells (5.56%). Percentage increases in crude protein content were 35.25% and 36.69% for *A. niger* and *T. viride* respectively. Both fungi reduced the crude fibre content of the shells significantly ($p < .05$), with *T. viride* causing a greater reduction of 14.88%. ANDGS and ANDGS had lower nitrogen-free extract contents (72.44% and 71.04% respectively) compared to the raw shells (74.21%). Fat content of the shells increased from 1.1 to 1.96% after biodegradation with *T. viride* but decreased to 0.88% for *A. niger*. Tannin and cyanogenic glycosides were completely depleted by both fungi. Oxalate content decreased while phytate content increased. Energy content of *T. viride*-degraded sample was higher than that of the raw sample though the difference

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was not statistically significant ($p>.05$). The findings show that biodegradation with *A. niger* and *T. viride* can enhance the feed potential of groundnut shells.

Keywords: Anti-nutrient, *Aspergillus niger*, feed potential, groundnut shell, nutrient composition, *Trichoderma viride*

Introduction

High cost of animal feed is the most important factor that affects profit margin in the livestock industry. About 70% of the expenditure of the animal production industry is said to be incurred on feed (Issaka, 2011; Demissie, 2022). Rising cost of commercial feeds in Nigeria has necessitated a search for affordable alternative local feed sources. This high cost of feeds is traced to scarcity of maize and soya beans which are the major raw materials for feed production. The use of maize in animal feeds as source of energy (carbohydrate) and soya beans as protein source is becoming increasingly unjustified in economic terms because of the grossly increasing cost of these feed ingredients (Abdollahi & Ravindran, 2021). Inadequate supply of feed ingredients like maize and soya bean is due to high competition between human and livestock for the commodities. Efforts are being made to replace the expensive feedstuffs with cheaper and more abundant unconventional feed materials in order to support the sustainability of animal production (El-Deek *et al.*, 2020) towards profit maximization. Large quantities of agricultural wastes which could serve as feed inputs are generated annually in Nigeria. These potential feed materials are mostly discarded and burnt, polluting the environment (Wuanor *et al.* 2018; Adejumo & Adebisi, 2020). Groundnut shells which are agricultural by-products are one of the most available biomass resources in the Nigeria, especially in the Northern Nigeria Ajayi & Lateef, 2023. This unique renewable energy resource has a high potential to be an alternative for animal feed (Millam *et al.*, 2020). High fibre content of and presence of anti-nutritional factors such as phytate, saponin, oxalate, cyanogenic glycosides and alkaloids in many agricultural waste products have, however, limited their use as animal feed (Onyimba *et al.*, 2019). The use of agricultural wastes such as groundnut shells as feed ingredients especially for non-ruminants

would require prior physical, chemical or biological treatment geared towards reduction of fibre content through breakdown of structural polysaccharides (Ajayi & Lateef, 2023), reduction of anti-nutritional factors, and enhancement of proximate composition. These will ultimately enhance the feed potential of the waste material. The biological pre-treatment of fibrous materials is not entirely new and biotechnological techniques are gradually being introduced into the field of livestock nutrition/biotechnology nutrition throughout the globe. If the huge amount of groundnut shells available in Nigeria could be recycled or converted to useful feedstuff, the level of environmental pollution will be reduced thereby improving human health (Sogbesan & Ekundayo, 2014) and increasing availability of animal feeds for optimal animal production. The aim of this study was to determine the abilities of *Aspergillus niger* and *Trichoderma viride* to enhance the feed potential of groundnut shells through biodegradation.

Materials and Methods

Collection and Preparation of Groundnut shells

Groundnut shells used in the experiment were sourced from groundnut farmers at Angwan Rukuba, in Jos North LGA of Plateau State, Nigeria. The shells were brushed free of soil particles, dried, and transported to the laboratory in clean moist free polythene bags. The groundnut shells were pulverized and dried at 60°C in a hot air oven after which they were stored in sterile dry glass jars.

Isolation and Identification of *Aspergillus niger* and *Trichoderma viride*

Dried pulverized groundnut shells (20 g) were transferred in triplicates into clean sterile conical flasks and moistened with 35 ml of sterile distilled water. A sterile spatula was used to mix the contents of the flasks and the mouths of the flasks were then covered with sterile cotton wool. The pulverized groundnut shells were allowed to ferment naturally at room temperature ($26\pm 2^\circ\text{C}$) over a period of 14 days with periodic stirring to improve aeration. Samples (1 g each) of the fermenting material were taken at weekly intervals and plated out on

malt extract agar (MEA) and on cellulose agar using the pour plate method. The plates were incubated at room temperature ($26\pm 2^{\circ}\text{C}$) for 5-7 days. After the incubation period, observed fungal colonies that had characteristic macroscopic morphological features of *Aspergillus niger* and *Trichoderma viride* were sub-cultured on malt extract agar and further observed under the microscope for typical features of *A. niger* and *T. viride*. Diagnostic features looked out for included nature of mycelia (septate or non septate, hyaline or coloured, smooth-walled or thick-walled), nature of fruiting body, nature of spores (smooth or thick-walled, spinulose or non spinulose), nature of conidiophore and phialides. The identities of *A. niger* and *T. viride* isolates were confirmed with the aid of identification manuals such as Domsch *et al.* (1980), Samson *et al.* (1984), and Pitt and Hockings (1997).

Effect of Biodegradation on Proximate, Anti-nutrient, and Energy Contents

Twenty grams of pulverized groundnut shells were placed in an improvised biodegradation vessel which consisted of a 250 ml conical flask with mouth plugged with a rubber bung. The rubber bung had two glass tubes running through it to allow the passage of air in and out of the flask. The outer end of the tubes were plugged with cotton wool and covered with aluminum foil. The biodegradation vessel containing pulverized groundnut shells was autoclaved for 15 minutes at 121°C and $1\text{kg}/\text{cm}^2$ pressure. The sterilized groundnut shell samples in the vessels were allowed to cool to room temperature. Separate vessels were provided for inoculation with each test organism. The sterile groundnut shell samples in the vessels were inoculated separately with two 5mm mycelial discs obtained from the margins of 4-day old cultures of *Aspergillus niger* and *Trichoderma viride*. The inoculated sample was mixed properly using pre-sterilized spatula. The aluminum foil covering the outer ends of the glass tubes were removed and the inoculated substrates were allowed to biodegrade aerobically at room temperature ($25\pm 2^{\circ}\text{C}$) over a period of seven days. The experiment was carried out in triplicates. At the end of the biodegradation period the biodegradation was arrested by drying the

samples in an oven at 60°C. The dried samples were properly mixed and stored in sterile dry bottles for subsequent analysis. Proximate composition, anti-nutrient content, and energy value of non-degraded and biodegraded groundnut shells were determined. Proximate composition was determined by the method of AOAC (2002) while anti-nutrient content was determined by the method adopted by Onyimba *et al.* (2019). Energy content was determined in line with FAO (2003) using Atwater factors of 4, 4 and 9 for protein, carbohydrate and fats respectively. It was calculated by adding up the product of Atwater factors against their respective energy parameters (protein, fats and carbohydrates). The effects of biodegradation with *A. niger* and *T. viride* on the proximate, anti-nutrient, and energy contents groundnut shells was determined by comparing the biodegraded and non-degraded groundnut shells in relation to the three parameters.

Statistical Analysis

The effects of biodegradation on proximate, anti-nutrient, and energy compositions of groundnut shells was statistically analysed using one-way analysis of variance (ANOVA) test with the aid of Microsoft excel version 2010 software. P-values less than 0.05 were considered significant. Means with significant differences were separated using Least Significant Difference (LSD).

Results

Effect of Biodegradation on Proximate Composition of Groundnut Shells

The protein content of groundnut shells biodegraded with *A. niger* and *T. viride* increased significantly ($P < .05$) by 35.25% (from 5.56 to 7.52%) and 36.69% (from 5.56 to 7.60%) respectively. Nitrogen-free extract content decreased from 74.21% to 72.44% and 71.04% for *A. niger* and *T. viride* respectively. Biodegradation brought about significant reductions in the crude fibre content of the groundnut shells with *T. viride* causing a greater reduction (16.98 to 14.78%). Fat content increased significantly (from 1.1 to 1.96%) for shells biodegraded with *Trichoderma viride* but decreased to 0.88% for *Aspergillus niger*-degraded shells. Details of the effects of biodegradation on the proximate composition of groundnut shells are presented in Figure 1.

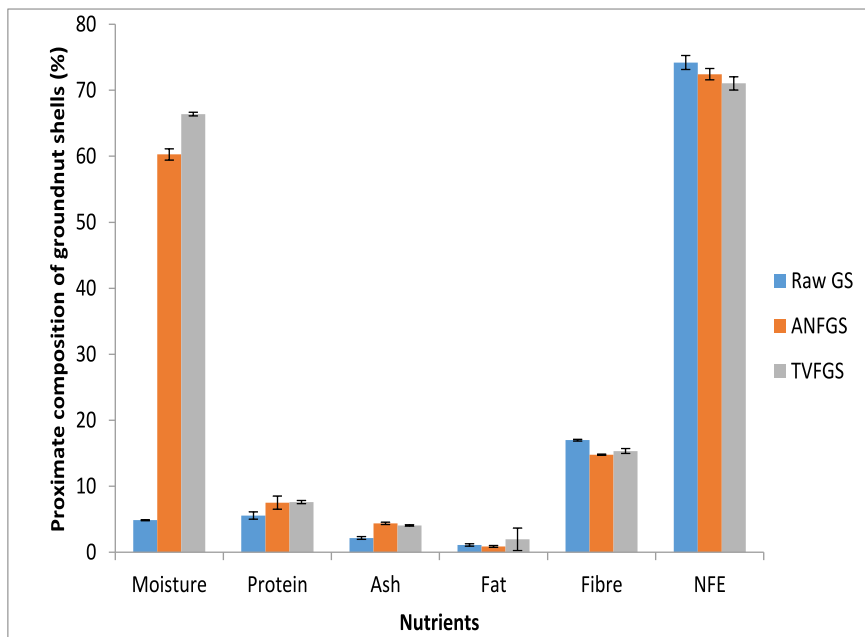


Figure 1: Effects of biodegradation with *A. niger* and *T. viride* on the Proximate Composition of Groundnut Shells

Key:

GS = Groundnut shells, ANDGS = *A. niger*-degraded groundnut shells,
 ANDGS = *T. viride*-degraded groundnut shells

Effect of Biodegradation on the Anti-nutrient Composition of Groundnut Shells

Figure 2 shows the effect of biodegradation on the anti-nutrient composition of groundnut shells. Biodegradation with *A. niger* and *T. viride* brought about significant reductions ($p < .05$) in oxalate contents of the shells with *T. viride* causing a greater reduction. No trace of tannin and cyanogenic glycosides was found in the groundnut shell samples biodegraded with both organisms. The phytate content of the biodegraded groundnut shell samples were higher than that of the non-degraded sample.

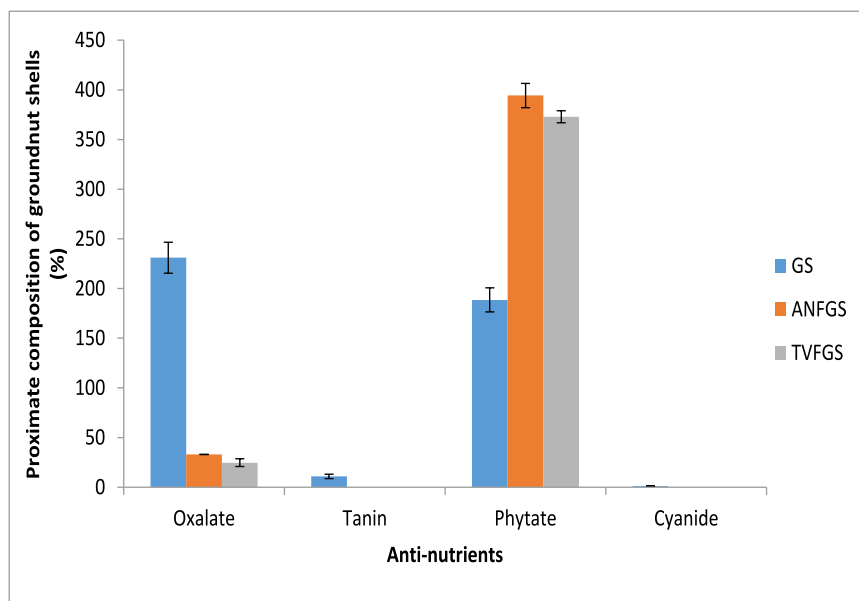


Figure 2: Effects of Biodegradation with *A. niger* and *T. viride* on the Anti-nutrient Composition of Groundnut Shells

Key: GS = Groundnut shells, ANDGS = *A. niger*-degraded groundnut shells
 ANDGS = *T. viride*-degraded groundnut shells

Effects of Biodegradation on the Energy Content of Groundnut Shells

The energy content of groundnut shells biodegraded with *T. viride* were found to increase by 1.76% (from 326.98 to 332.2 Kcal/100g) while that of the shells degraded with *A. niger* decreased insignificantly ($p > .05$) by 0.78% (from 326.98kcal/100g to 326.44 kcal/100g).

Discussion

Effect of Biodegradation on Proximate Composition of Groundnut Shells.

The study showed that biodegradation of groundnut shells with *Aspergillus niger* and *Trichoderma viride* brought about significant changes in the various nutritional constituents of the shells. Crude fibre content of the shells was reduced significantly by both fungi, *T. viride* having caused the greater reduction of 13%. The reduction of crude fibre observed in this study is in conformity with the findings of Joseph *et al.* (2011) who reported a reduction in the crude fibre contents of fermented rice straw and groundnut shells. Several authors have also reported reductions in crude fibre contents of fibrous agricultural wastes due to fungal biodegradation (Onyimba *et al.*, 2014; Abosiada *et al.*, 2017). The decrease in fibre contents of groundnut shells observed in this study was probably due to fibrolytic activity of *A. niger* and *T. viride* which are known to possess fibre degrading ability (Aliyu and Inuwa, 2015). Since *A. niger* and *T. viride* are not major lignin degraders, the lignin content of the substrate is expected to be largely intact while cellulose and hemicellulose contents would have been depleted. This decrease in fibre content is desirable considering that high levels of fibre may not be tolerable especially for several monogastric livestock animals which are normally limited in ability to degrade fibre (Lindberg, 2014). Fibre content of biodegraded groundnut shells is not likely to be a problem for ruminants.

Biodegraded groundnut shells had higher crude protein content compared to the raw shells. The observed increase in crude protein content agrees with the findings of Abosiada *et al.* (2017) who reported increases in the protein contents of peels of mango, apple, banana, and tomato subjected to solid state fermentation with *Trichoderma reesei*. Aliyu and Inuwa (2015) also obtained a similar increase in crude protein content of groundnut husks fermented with *T. viride* and *A. niger*. The observed higher crude protein content of biodegraded groundnut shells in the present study was probably due to

bioconversion of carbohydrates in the fungal colonized shells into mycelial protein. Nester *et al.* (1973) explained that there is a key reaction that links energy metabolism to biosynthesis and that organic acids formed from glucose metabolism react with ammonia or the ammonium ion to yield amino acids. Such amino acids are subsequently converted to protein. Protein is an essential key ingredient of animal feeds. It is needed for animal growth, body maintenance, reproduction, and maximal output of animal products including milk, egg and wool. Though the crude protein contents of the degraded groundnut shells remained lower than the requirement for most monogastric livestock animals, the nutritive value of the shells have been enhanced in terms of protein content. This reduces the amount of supplementary protein that would be required by animals feeding on the biodegraded shells.

Biodegradation with *T. viride* and *A. niger* led to decreases in the nitrogen-free extract (NFE) content of the shells. The lower NFE values observed could have been due to metabolism of simple sugars for energy generation and also as a result of use of products of sugar metabolism for protein synthesis. This finding is in conformity with that of Iyayi and Aderolu (2014) who reported a decline in the soluble carbohydrate values of agro-industrial byproducts fermented with species of *Aspergillus* and *Penicilium* and that of Onyimba *et al.* (2015) who reported a decrease in the nitrogen-free extract content of sweet potato leaves fermented with a co-culture of *Chaetomium globosum* and *Saccharomyces cerevisiae*. It is however not in conformity with the findings of Wuanor *et al.* (2018) in which the NFE content of groundnut shells biodegraded by *Pleurotus tuber regium* were found to increase. Variation in NFE contents could arise from differences in fermentation time and fungus used for the biodegradation.

The lipid content of ANDGS increased significantly. This finding agrees with that of Wuanor *et al.* (2018) who reported an increase in ether extract content of groundnut shells subjected to fungal

biodegradation. The increase in lipid content of the biodegraded shells may have been caused by depletion of the contents of other proximate components which could result in an increase in the percentage lipid content of the substrate. The lipid content of the fungal mycelium produced may have also contributed to the increase in lipid content. The lipid content of ANDGS however decreased by 20%. This reduction in lipid content could have resulted from a possible use of lipid by the fungus as a secondary source of energy. It could also have resulted from changes in the values of other proximate components of the shells which could in turn affect the percentage lipid content.

The moisture content of ANDGS and TVDGS (60.27% and 64.39% respectively) were found to be higher than that of the raw shells. The higher moisture content of the degraded materials could have resulted from the addition of water to the pulverised shells before biodegradation. Ojokoh *et al.* (2015) similarly reported increases in the moisture content of pearl millet-acha flour blends after fermentation. In the findings of Nejad *et al.* (2017), similar moisture content levels in total mixed rations (TMRs) fed Corriedale ewes did not negatively affect performance parameters. Ash contents of ANDGS and TVDGS were significantly higher ($P < 0.05$) than that of the non-degraded shells. These increments in ash content could have resulted from enrichment of the mineral content of the groundnut shell substrate by the fungal mycelia. Similar findings have been reported by Wuanor *et al.* (2018) for groundnut shells biodegraded by *Pleurotus tuber regium*. Akinfemi *et al.* (2009) in an earlier study equally reported that biodegradation of maize straw by species of white rot fungi brought about increases in the ash content of the straw.

The contents of tannin and cyanogenic glycoside of the groundnut shells were found to be completely depleted by both *A. niger* and *T. viride*. These findings are in conformity with that of Terefe *et al.* (2021) who reported a significant reduction in the tannin content of maize flour fermented with *L. plantarum* and *S. cerevisiae* and that of Iwuoha *et al.* (2013) who reported a reduction in the cyanogenic glycoside content of

fermented cassava tubers. Tannins are known to impart astringent taste to feed which causes depression in feed intake by animals and this results in poor growth. Tannins reduce feed digestibility and also bind to proteins making them not to be readily available in the diet (Hassan *et al.*, 2020). The complete removal of tannin and cyanogenic glycosides from the groundnut shells by *A. niger* and *T. viride* implies an improvement in terms of feed quality of the shells. The phytate content of the groundnut shells were found to increase after biodegradation. This could have been due to synthesis of phytate during fermentation process. The observed increase in phytate content is at variance with the findings of Aliyu and Inuwa (2015) who reported a significant reduction in the phytate content of groundnut husks after biodegradation with *A. niger* and *T. viride* and also at variance with the findings of Ogodo *et al.* (2019) who also reported a reduction in the phytate content of maize flour fermented with selected species of lactic acid bacteria. Phytate interferes with livestock animal performance by reducing amino acid digestibility and mineral absorption, and by increasing endogenous losses. The negative effects of phytate could be reduced by addition of phytase enzyme to feed.

Conclusion

Biodegradation of groundnut shells by *A. niger* and *T. viride* enhanced the crude protein and fat contents of the shells and reduced the levels of crude fibre, tannins, cyanogenic glycoside and oxalate. Both fungi were thus able to improve the nutritional quality of the shells in a number of aspects, with *T. viride* being the more effective fungus. Apart from the observed increase in phytate content of the biodegraded shells, the nutritive value of the shells was enhanced by the biodegradation process thereby increasing the potential for use of the biodegraded shells as animal feed or feed supplement. Actual use of biodegraded groundnut shells for feed purposes would however depend on results of feeding trials with selected animals.

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