AJBS
Asian Journal of
Biological
Sciences

OPEN ACCESS

Biodegradation of Leguminous Husks into High Protein Feed Products Using Oyster Mushrooms (*Pleurotus ostreatus*)

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ABSTRACT

Background and Objective: High cost of conventional feedstuff is a major constraint to commercial livestock production in Nigeria. Leguminous crop residues which are abundant and cheap can fill the gap but are of very low nutrient quality. This study investigated the effect of *P. ostreatus* on three leguminous residues namely, groundnut shell (GS), cowpea husk (CH) and bambara nut shells (BS) with sawdust (SD) as the control. **Materials and Methods:** The 500 g of the different moist substrates which had been previously dried and milled into smaller particle sizes of at least 2 mm in diameter, were inoculated with 10 g of *P. ostreatus* spawn which were covered with transparent cellophane bags and placed in a dark room for 5 weeks to allow optimum conditions for colonization of the substrate. The samples were replicated 3 times. Subsequently, samples from each replicate were bulked, oven-dried and milled for chemical analysis. **Results:** The proximate analysis before and after the treatment showed an increase in nutrient quality parameters, crude fibre 42.05 vs 30.95%, 42.45 vs 37.90%, 52.32 vs 28.8%, 68.35 vs 59.5% for (GS), (CH), (BS) and (SD), respectively, crude protein 9.24 vs 18.08. 9.45% vs, 14.18, 9.30 vs 11.03% and 1.93 vs 2.63% for (GS), (CH), (BS) and (SD), respectively, ash content 10.60 vs 8.30%, 7.80 vs 12.56%, 10.90 vs and 13.50%, 0.5 vs 1.5% for (GS), (CH), (BS) and (SD), respectively, fat and oil 12.26 vs 1.90%, 5.70 vs 18.52%, 3.78 vs 3.78% and 7.04 vs 1.88% for (GS), (CH), (BS) and (SD), respectively. **Conclusion:** The mushroom variety *Pleurotus ostreatus*, could be used to enhance the nutritive quality of the crop residues under study.

KEYWORDS

Biodegradation, bambara nuts, groundnut shells, cowpea husk, fibrous feeds, lignocellulose, oyster mushroom, *Pleurotus ostreatus*

ISSN: 1996-3351 (Print)
https://doi.org/10.3923/ajbs.2023.344.350

Received: 29 Mar. 2023
Accepted: 25 Aug. 2023
Published: 30 Sep. 2023

INTRODUCTION

High feed costs and scarcity of conventional feedstuffs such as groundnut cake, soya bean cake, palm kernel cake, blood meal and fish meal are pushing researchers to find low-cost alternatives. In the heat of the raging competition between man and animals for grain and pulses, prices of hitherto cheaper agro-industrial cereal by-products have also increased. One way out is to seek ways of adding value to previously neglected by-products in the agricultural processing value chain especially high-fibre lignocellulosic materials from crop residues, particularly for ruminant feeding.

Crop residues are the postharvest materials that remain after the harvest of the main crop for human consumption. They include fibrous parts of cereals, legumes, roots and tubers (cassava, sweet potato and yam peels etc.). Although, they have lower feed value when compared to the main crop, they are valuable for ruminant feeding, especially during periods of feed scarcity. Wastes from leguminous crops are most abundant and comprise 50% of all biomass with an estimated annual global production of amounts to about 200×10^9 tons/year, of which over 90% is lignocelluloses[1]. These residues sometimes constitute a nuisance to the environment. It is often recommended for use in soil improvement and as compost, but sometimes the quantity can be unmanageable leading to its destruction by burning, which is an environmentally unsustainable practice. The digestibility of lignocellulosic materials is very low because of the inherent lignin in the cellulose and hemicellulose matrix. Cell wall lignifications of crop residues have been reported as a major factor that limits the availability of cell wall structural carbohydrates for animal utilization[2]. The effect of lignin on cellulose degradation by cellulase and its role in cellulose recovery from organisms during hydrolysis of lignocellulosic reveal that lignin had an inhibitory effect on rumen micro-organisms[3].

One of the strategies to improve the nutritive quality of such crop residues is the use of fungi such as edible mushrooms (*Pleurotus oestreatus)* that will not only reduce the fibre but also help in obtaining protein-rich substrates. Edible mushrooms can bio-convert a wide variety of lignocellulosic materials due to the secretion of extracellular enzymes[4]. The natural ability of some fungi, particularly some species of *Pleurotus ostreatus* to upgrade lignocellulosic materials into animal feeds is well documented in the literature[5]. Attempts had been made to identify species of white-rot fungi for their ability to grow on straws to improve their nutritive value. In Nigeria, the appropriate method of recycling these wastes into forms that will be useful to the animals has generated interest in recent times. The white-rot fungi *P. ostrea*tus and *P. pulmonarius* are wood-decaying basidiomycetes which are capable of degrading not only lignin but also variable recalcitrant environmental pollutants due to their ability to secrete ligninolytic enzymes such as lignin peroxidase, manganese peroxidase and laccases which aid in the degradation process[6]. Previous studies have shown the feasibility of using agricultural wastes to produce animal feed[5] and as substrates for mushroom production[7]. However, studies on the comparative effect of *Pleurotus ostreatus* on these three leguminous husks have not been reported extensively hence the need for this study. Groundnut, cowpea and Bambara nuts are of particular interest in the agricultural economy of Nigeria due to their value as cash crops and the huge tonnage produced and wastes generated annually from their processing.

Bambara groundnut (*Vigna subterranean* (L.) Verdc.) is a pulse with subterranean fruit–set and is cultivated by smallholders over much of semi-arid Africa. The crop is a legume species of African origin and is drought tolerant and widespread south of the Sahara[8]. Groundnut shell has great potential for commercial use. It is used as a fuel, filler in cattle feed, hard particleboard, cork substitute and activated carbon. In the past, peanut skins have been viewed as a low-value byproduct of peanut processing and roasting. However, recent studies have shown that these byproducts contain compounds including isoflavones, isorhamnetin, epicatechin, catechin, resveratrol and quercetin. Other recent studies on groundnut shells have found their importance in the production of substances such as ethanol and enzymes (tannase, lacasse cellulase). The content of crude fibre is high, close to 60% hence its potential as a good source of fibre for ruminants. It has been reported to contain cellulose 65.7%, carbohydrates 21.2%, proteins 7.3% and minerals 4.5%[9]. Cowpea (*Vigna unguiculata*) is a major grain legume grown by small-scale farmers in the arid and semi-arid regions of West Africa. Cowpea seed hull (husk or hull in botany is the outer shell or coating of a seed) *i*s a crop residue that is available in Nigeria in large quantities. It is a post-threshing residue that though high in fibre, has found use in ruminant nutrition. The objective of this study was to determine the effect of Oyster mushrooms (*Pleurotus ostreatus)* on the chemical composition of three leguminous crop residues.

MATERIALS AND METHODS

Experimental site: The research was carried out at the poultry unit of the School of Agriculture and Agricultural Technology (SAAT) Teaching and Research Farm of the Federal University of Technology Owerri, Imo State for 3 months, during April and June 2016. Owerri is located in the humid Southeastern Agricultural Zone of Nigeria. It is located at an altitude of 90 cm above sea level, the mean annual rainfall is 2500 mm with mean temperature and humidity of 26.5-27°C and 70-80%, respectively.

Source of experimental materials: The cowpea husk, weighing about 25 kg, was purchased from roughage feed dealers at the Obinze, livestock market, in Imo State, while the bambara husk and groundnut husk were purchased at Orba-Nsukka in Enugu State. The materials were sun-dried and after sun-drying milled in a hammer mill to reduce the particle size and to increase the surface area available for the enzymatic digestion of cellulose by the fungus. The sporophores of *Pleurotus ostreatus* growing in the wild were collected and tissue culture at the Department of Crop Science, University of Port Harcourt Choba, Rivers State to obtain fungal mycelia. The pure culture obtained was maintained in a bottle covered with aluminium foil.

Processing the experimental materials: The groundnut shell was milled with a hammer mill. The various substrates were prepared by first autoclaving locally using a metal drum containing little water, separated from the materials by wire gauze. The metal drum was heated for about an hour, the steam was allowed to sterilize the substrate. The materials were spread lightly to cool. Subsequently, 500 g of each substrate was put in each of the plastic bowls. Each treatment was replicated three times. Each sample used was inoculated at the centre of the substrate with mycelia and covered immediately. They were kept in an enclosed room where the average temperature was between 24-26°C and 90% relative humidity. After 5 weeks of inoculation, the samples were harvested by autoclaving again to terminate the mycelia growth. Samples of the biodegraded husks were oven-dried to constant weight for chemical analysis.

Chemical analysis: Nitrogen (N) content of the husks was determined by the standard Kjeldahl method and the amount of crude protein was calculated as (Nx6.25). Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and crude fiber (CF) were assessed using the standard methods proposed by van Soest *et al.*[10]. Concentrations of Ca, Mg and K. of feedstuffs were determined by Atomic Absorptions spectrophotometer (GBC 908AA, GBA Australia).

RESULTS AND DISCUSSION

The results of the proximate composition and elemental analysis are presented in Table 1 and 2 respectively. The increase in the content of crude protein among the substrates after biodegradation ranged from 21% in bambara nuts to 96% in groundnut. The change in the contents of fats and oil was not consistent. While cowpea increased by 200%, groundnut and saw dust decreased by 600 and 274%, respectively, while bambara nut was stable. Furthermore, the ash content increased by 61% in cowpea, 24% in bambara nuts and 200% in sawdust while showing a 23% decline in ground nuts. The fibre contents declined by 11% in cowpea, 21% in groundnut, 45% in bambara nut and 13% in sawdust. Also,

Table 1: Comparative proximate analysis (% DM) of cowpea and other leguminous crop residues before and after biodegradation with oyster mushroom, (*Pluerotus oestratus*)

	Cowpea husk (%)		Groundnut shell (%)		Bambara nut shell (%)		Saw dust(%)	
Parameter	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
Crude Protein	9.45	14.8	9.24	18.08	9.30	11.03	1.93	2.63
Fats and oil	5.71	18.52	12.26	1.9	3.78	3.78	7.04	1.88
Moisture	12.7	11.08	10.7	27.56	5.9	28.25	8.14	6.26
Ash	7.8	12.56	10.6	8.3	10.9	13.5	0.5	1.5
Crude fibre	42.45	37.9	42.05	30.95	52.32	28.8	68.35	59.5
Carbohydrate	21.89	5.00	15.15	13.21	18	14.64	14.04	28.23
Total	100	100	100	100	100	100	100	100

Table 2: Elemental analysis (%) of leguminous crop residues before and after biodegradation with oyster mushroom, *Pluerotus oestratus*

	Cowpea husk (%)		Groundnut shell (%)		Bambara nut shell (%)		
Parameter	Untreated	Treated	Untreated	Treated	Untreated	Treated	SEM
Ca	2.23^{d}	12.20^{a}	2.73^{c}	1.69^{e}	3.30^{b}	2.23^{d}	0.03
K	0.27^{cd}	0.84^{a}	0.53^{b}	0.41^{bc}	0.41^{bc}	0.14^{d}	0.04
Na	0.12^{f}	105.00^{c}	37.00^{d}	108.00^{b}	4.00^{e}	116.00^{a}	0.01
Mn	0.03^{c}	0.43^{a}	Nil	0.09^{b}	Nil	0.42^{a}	0.01
Cu	0.10	0.10	Nil	Nil	Nil	0.10	0.00
Fe	0.13^{e}	2.53^{b}	2.32^{c}	2.55^{b}	0.54^{d}	38.10^{a}	0.02
Zn	0.22^{a}	0.07^{bc}	0.05^{bc}	0.14^{ab}	Nil	0.08^{bc}	0.03
Ni	0.44^{b}	0.88^{a}	Nil	0.10^{c}	Nil	0.03^{c}	0.02
Mg	1.52^{f}	124.00^{a}	18.00^{d}	23.00^{c}	16.00^{e}	47.50^{b}	0.02
Pb	0.30^{a}	0.10^{b}	Nil	Nil	Nil	Nil	0.00

*Mean values along the same rows with different superscripts are significantly different ($p<0.05$), SEM: Standard error of the mean
Ca: Calcium, K: Pottasium, Na: Sodium, Mn: Manganese, Cu: Copper: Fe: Iron, Zn: Zinc, Ni: Nickel, Mg: Magnesium and Pb: Lead

the contents of non-fibre carbohydrates declined by 337% in cowpea, 13% in groundnut and 19% in bambara nut but increased by 100% in sawdust. The increased content of crude protein in the leguminous crop residues treated with oyster mushrooms could be a result of the increase in fungal biomass[11] and the mobilisation of previously unavailable nitrogen from the cell wall matrix. The major part of the protein in low nitrogen-containing roughage is mostly associated with the cell walls which have low digestibility[12]. Hence, straws subjected to fungi degradation contained more free sugars, more protein with less cellulose and lignin and increased content of ash compared to the starting material. The crude protein content of groundnut shells increased by 48%, which is in agreement with the report of Akinyele *et al.*[13], who obtained an increase in the crude protein (CP) content with *Volvanella volvacea* treated agro-waste and that of Kinfemi *et al.*[14] when cowpea shells were treated with *P. ostreatus* and *P. pulmonarius*. Although some strains of fungi are reported to degrade and consume cellulose, protein and other organic matter during bio-degradation, this has not been reported for *Pleurotus ostreatus*. In most of the cases reported in this study, there was a general increase in the contents of crude protein and ash and a reduction in the contents of crude fibre. The crude fibre constitutes on average 30-35% of plant biomass. n this study, the crude fibre (CF) content of *P. ostreatus* treated samples of cowpea husk (CH) and saw dust (SD) respectively, decreased significantly in line with the findings of Kinfemi *et al.*[14].

In this study, the ether extract (EE) or fat and oil increased in the treated samples except for sawdust and groundnut shells which might be attributed to the production of some fatty acids by the micro-organisms in the cells following an increase in the microbial mass. This is in agreement with the reports by Olagunju *et al.*[15] that *P. ostreatus* treatment on corn stover increased crude protein (58.5%), ash (25. 32%) and non-fibre carbohydrates (118%). Also, Asmare[16] reported that mushrooms utilize carbon and energy source in the substrate for metabolism to break down lignocellulose structures to release cellulose and improve the digestibility of nutrients thereby enhancing the feeding value of lignocellulosic.

The result from the analyses in this study revealed that the contents of ash from cowpea husk (CH), bambara nut shells (BS) and sawdust (SD) increased after treatment with *P. ostreatus* except for groundnut shells which decreased by 22%. The ash contents in (CH) and (SD) increased by 62 and 200%, respectively. The differences in the values of the treated and untreated substrates could be attributed to selective absorption or decay by the fungi as described by Shrivastava *et al.*[17]. These increments in the ash values of CH and SD could be attributed to the fact that the mycelia of the fungus had enriched the mineral content of the substrate. Similar results were also reported by other workers such as Alemawor *et al.*[18] and Asmare[16], who found various levels of increases in nutrient content during *Pleurotus ostreatus* fermentation of corn cobs and cocoa pod husk (CPH), respectively.

Changes in mineral content following the fermentation of the various substrates with *P. ostreatus* evaluated by the atomic absorption spectrophotometer readings were evaluated and there were selective increases or decreases in the amounts of the various micro and macro elements. It could be inferred that the decreased mineral contents resulted from the uptake by the fungi resulting in its depletion since minerals are important in fungal nutrition. The trend of increase and decrease of the minerals was not consistent. However, the various minerals that recorded higher contents could have resulted from the increased accumulation of the elements by the fungal biomass[11]. The increased content of the following mineral elements Na, Mn, Ca, Fe, Ni, Mg and K in the treated cowpea shells could mean that the multiplication of the fungal biomass resulted in the enhanced production of the given mineral elements. This is expected since Pleurotus species have been reported as rich sources of protein, minerals (P, Ca, Fe, K and Na) and vitamins, thiamine, riboflavin, folic acid and niacin[19]. Also, Asmare[16], reported that mushrooms have a richer supply of minerals than many meat products and are two times higher than vegetables. Additionally, the fruiting bodies of mushrooms contain about 10% ash on a dry matter basis (However, a decreased level of Zn and Pb, as was the case, could mean that the fungi might be utilizing these minerals for their metabolism. This might imply that oyster mushrooms may be useful in decontamination of polluted sites. Similarly, groundnut shell had increased Cu, Fe, Zn, Ni, Mg, Pb, Na and Mn but a decreased Ca and K content while Bambara nut had an increase in Na, Mn, Cu, Fe, Zn, Ni, Mg, Pb and decreased in Ca and K content. The decrease in values of some of the macro elements is at variance with findings by other authors such as Asmare[16].

According to Mahesh and Mohini[12] use of biological treatments for improving the feeding value of low-quality fibrous crop residues leads to organic matter losses through microbial degradation. The inevitable organic matter losses during biological treatments imply that an increased organic matter (OM) digestibility is needed to compensate for the losses. Hence, solid-state fermentation (SSF) for 6-8 days has been recommended as the maximum time of fermentation to reduce dry matter loss[20]. However, Asmare[16] recommended an optimum fermentation period of six weeks for the biodegradation of any substrate. Although improvement in the nutritional worth of biologically treated crop residues is achieved, many are not economical and the process has not yet been optimized under field conditions. Hence, the possible future of the technology should focus on the isolation and identification of selective and highly lignin-lytic fungi in nature and cultivating them for the commercial production of ligninase enzyme[21].

Furthermore, genetic manipulation of the lignin-lytic fungus such that only lignin is degraded without any changes in cell wall carbohydrates should be developed and adopted. Once the proven fungus is identified, its potential to upgrade (enhancing digestibility) various agro by-products (husks, straw, stovers, bagasse and other fibrous lignocellulosics) that are traditionally used as livestock feeds should be considered. In addition, these techniques should be low-cost such that resource-poor crop-livestock farmers can easily adopt them. Overall, biodegraded crop residues are reported to reduce methane gas emissions with significant implications for sustainable environmental management and global warming.

CONCLUSION

The use of oyster mushrooms in the biodegradation of leguminous crop residues such as cowpea, groundnuts and bambara nut husks improved the nutrient content of protein and ash of poor-quality leguminous crop residues while reducing the contents of crude fibre and carbohydrates.

SIGNIFICANCE STATEMENT

This study discovered that oyster mushrooms are useful agents in the biodegradation of fibrous crop residues into higher-quality feedstuff than the starting material. The selective absorption of certain mineral elements in preference to the others could provide more insight into the theory of mineral uptake and metabolism of oyster mushrooms. More research is needed to evaluate the prospect of the use of oyster mushrooms as a candidate in the bioremediation of polluted sites.

ACKNOWLEDGMENT

The authors are grateful to the Management and Staff of the School of Agriculture and Agricultural Technology for the provision of space and facilities for sample analysis.

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