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BIOCHEMICAL CHANGES OF SOME WASTE AGRICULTURAL RESIDUES AFTER SOLID STATE FERMENTATION

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ABSTRACT

Four waste agricultural residues were screened to determine their suitability for solid – substrate fermentation and nutritional enrichment in a completely randomized design and 'T' test experiments. There was an increase in the crude protein content of the fermented substrates compared with the unfermented substrate (cassava waste, rice husk, groundnut shell and cowpea husk) and protein enrichment was highest with rice husk followed by cowpea husk and lowest for cassava wastes. The ether extract composition of substrates fermented with the fungus increased from 7.00 to 10.50% (Rice husk). 5.00 to 10.53% (cassava waste). 0.9 to 2.5% (groundnut shell) and 3.50 to 5.90% (cowpea husk). The crude fibre and other fibre fractions (Acid detergent fibre and lignin) of the fermented substrates (cassava waste, rice husk, cowpea husk and groundnut shell) showed significant ($p < 0.05$) reduction compared to the unfermented substrates. Significant differences ($P < 0.05$) among fermented substrates were found for the proximate composition and fibre fractions. This study demonstrated that solid-state fermention of some waste agricultural residues (rice husk, cowpea husk, groundnut shell and cassava wastes) with *Aspergillus niger* increased the crude protein and ether extract contents while the crude fibre and other fibre fractions (lignin and acid detergent fibre) decreased significantly ($P < 0.05$), all of which are limiting nutrients in livestock (ruminant) nutrition.

KEYWORDS: *Aspergillus niger*, waste agricultural residues, proximate composition, fibre fraction.

INTRODUCTION

The limiting nutrient qualities of waste agricultural residues include low protein and high fibre contents as well as poor digestibility due to a complex bond (lignocellulose) formed by three polymers thus: cellulose, hemicellulose and lignin (Masonet al., 1993). The protein in these waste agricultural residues ranges from 5.3% (cassava waste) to 11.4% (rice husk) (Belewu, 1992). Belewu and Akinladenu, 1998).

Rice husk constitute about 20 – 25% of the unmilled rice and are vital by - product of the rice industry (BAE. 1974). White (1966) found that it took more than two hundred hours (200hrs) after feeding of ground rice husk to be 95% eliminated and that it passes through the tract of cattle more slowly than ground hay. Belewu and Okhawere (1998) reported improved performance on goat fed fungus (*Trichoderma harzanium*) treated rice husk compared to the untreated rice husk.

Groundnut which is indigenous to Tropical Africa has shell which contains 6.4% crude fat,7.2% protein and 5.8% crude fibre. However Belewu and Okhawere (1998) reported that the protein content of waste agricultural residues can be improved by treated with *Aspergilus niger*.

Cassava waste (consisting of the rind, peel and pulp and small tubers) which constitute by- product of gari processing industry is rich in energy but poor in protein. Inoculation of the waste with *Aspergillus niger* cultures and later incubated in fermentation travs recorded increasing crude protein content (5 – 48%)(Ngugen and Ngugen, 1992).

To enhance the potentials of these waste agricultural residues (cassava waste, rice husk, groundnut shell and cowpea husk) as a fibre feeding stuffs and more importantly as an alternative to maize, sweet potato, sorghum, millet and cassava pulp in the tropical environment, simple microbial biotechnology technique was used which involved the application of amylolytic exhibiting fungus (*Aspergilus niger*) in the protein enrichment of starchy and high fibre substrates.

However, protein enrichment of sweet potato by solid fermentation using amylolytic yeast or filamentous fungi are well documented (Yang., 1988; Yang and Eriksson, 1992: Abu et al 1999). Information on the effect of *Aspergillus niger* on cassava waste, rice husk groundnut shell and cowpea husk are not well elucidated in literature, this may be because most of these waste agricultural residues are indigenous to developing countries. Hence, the posterity of this study was to culture *Aspergillus niger* on some waste agricultural residues (cassava waste rice husk, cowpea husk and groundnut shell) and then screen the most nutritional enriched substrate for future use in the nutrition of ruminant animals.

MATERIALS AND METHODS

Fungus Used

Aspergillus niger used for the experiment was isolated from the soil through serial dilutions, identified and characterized accordingly. Stock culture of this fungus was maintained on potato dextrose agar containing in a Maccarthey bottle and stored at 4°C in a refrigerator.

Substrates Used

Cassava waste was obtained from cassava processing center, Ilorin. It was sun dried to 5 -6 % moisture content, milled and packed into polypropylene bags which were later sterilized by autoclaying at 121°C, 15kg/cm^2 for 15 minutes. It was allowed to cool and then inoculated with the inoculum (*Aspergillus niger*). Other substrates (rice husk, groundnut shell and cowpea husk) were treated as aforementioned for cassava waste.

Inoculation and incubation

The spores of *Aspergillus niger* were harvested with Tween 80 solution (10ml, 0.01% v/v) and adjusted to $10^7 - 10^8$ spores per ml with sterile water. Each bag (50g) was inoculated with 5ml of the spore suspension containing 10^7 spores per ml of each microorganism. The control treatment containing the substrate without the inoculum was separately prepared and inoculated with the inoculated substrates at ambient temperature. In about 7 days the fungus covered the substrates. The fermented substrates were later dried in a forced air laboratory oven to terminate the growth of the fungus and also dried the sample.

Physical and Chemical Characteristics

(i) Water Solubility

About 0.5g of the substrate sample was dissolved in 10m1 of distilled water. It was allowed to stand in water bath at

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38° C for 4 hours. The hydrolyzed solution of each at the end of 4 hours was filtered through Whatman filter paper No. 1. The residue was washed twice with distilled water and dried in oven for 16 hours. This was later weighed again and the percentage difference between this value and the initial weight is the water – soluble content.

(ii) **Ethanol Solubility**

It was determined by taken 10ml portion of 80% weight for volume ethanol and added to 1gm sample of treated and untreated samples in a 10ml conical flask. This was later boiled for 10minutes with continuous stirring. The mixture was then filtered with Whatman No. 1 filter paper. The process was repeated three times after which the residue was oven dried at 105°C for 16 hours and weighed. The percentage difference between the value and the initial weight was taken as the soluble content.

Particle Size

25g of each sample from treated and untreated cassava waste, rice husk, groundnut shell, was placed on sieve of pore sizes of 2.00mm. 1.00mm and 0.5mm. The sieve was shaken for 10minutes and the content was slightly stirred and shaken for another 5 minutes. The residue of each sieve was carefully removed with the aid of a brush and weighed. The procedure was replicated thrice and the mean value determined.

Chemical analysis

The fungus treated and untreated samples were subjected to proximate analysis according to the standard method of A.O.A.C. (1985) while the fibre fractions (ADF and lignin) were determined using the method of Goering and VanSoest (1970).

Statistical analysis

Data collected were subjected to student parametric 'T' test and completely randomized design model while treatment means were separated by Duncan (1955) multiple range test.

RESULTS AND DISCUSSION

The physical characteristics (ethanol and water solubility) of the fermented and unfermented samples showed similar results ($p > 0.05$) (Table 1). While significant variations were recorded for both the particle size and water content of the substrates (Tables 2 and 3). There was a reduction in the particle size of the fermented substrates compared to the unfermented samples due probably to the action of the fungus on the substrates. The data on the proximate and fibre fractions of the unfermented and fermented substrates are shown in Table 2. The fungus, *Aspergillus niger* increased the crude protein and ether extract contents, these were especially higher in rice husk and groundnut shell. The higher crude protein content reported here could be due probably to the addition of fungal protein during the fermentation process. This was in agreement with the previous studies (Yang et al., 1993, Abu et al., 1999). Since no external source of nitrogen was supplied in this study, there could probably be the conversion of some of the plant protein or other nitrogenous compounds into microbial protein. Protein build up was about 164.6% in cassava waste compared to 17.78 and 24.76% of cowpea ad rice husk respectively. The increase in the ether extract content of the fermented substrates agreed with the work of weete and Ghandi (1992) who reported similar trend for Oleaginous filamentous fungi (*Mortierella isabellian, Mucor Cincinelloides* and *Pencillin Spinulosum*) all of which are capable of producing 65% of their dry weight as lipids (Abu *et al.*, 1999).

Table 1: Physical Characteristics of the fermented and unfermented Waste Agricultural residues+.

	Ethanol	Water	Parture size		
Solubility	Solubility	2mm	1mm	0.5mm	
Rice husk					
Unfermented	0.65	5.63	23.60	0.90	0.50
Fermented	0.62	8.75	21.80	1.0	2.20
Significance	NS	*	*	NS	*
Cassava Waste					
Unfermented	0.68	10.63	24.20	0.40	0.40
Fermented	0.62	8.62	22.20	1.80	1.10
Significance	NS	*	*	*	*
Groundnut shell					
Unfermented	0.66	6.25	24.0	0.90	0.50
Fermented	0.63	4.70	23.0	1.40	0.60
Significance	NS	*	*	*	*
Cowpea shell					
Unfermented	0.58	10.00	23.0	1.00	1.00
Fermented	0.56	8.25	21.00	2.0	2.00
Significance	NS	*	*	*	*

\+ = T test

NS = Not Significant (P>0.05)

* = Significant at P>0.05

Table 2: Proximate Composition of the Unfermented and Fermented Waste Agricultural residues (%)

Rice husk	DM	CP	CF	EE	Ash	ADF	LIGNIN
Unfermented	94.37	11.38	60.00	7.00	2.10	60.40	56.20
Fermented	91.20	14.20	52.75	10.50	4.30	49.70	42.60
Significance	*	*	*	*	*	*	*
Cassava Waste							
Unfermented	89.37	3.25	87.50	5.00	4.30	84.20	52.40
Fermented	95.56	8.60	45.00	10.53	6.80	58.30	39.50
Significance	NS	*	*	*	*	*	*
Groundnut shell							
Unfermented	93.75	8.00	37.50	0.90	10.02	53.30	45.00
Fermented	95.30	10.30	30.21	2.50	12.75	46.15	41.50
Significance	NS	*	*	*	*	*	*
Cowpea shell							
Unfermented	90.00	9.00	55.00	3.50	2.58	84.50	61.50
Fermented	91.75	10.60	42.50	5.90	5.30	56.60	47.23
Significance	NS	*	*	*	*	*	*

\+ = T test
NS = Not Significant (P>0.05)
• = Significant at P>0.05

Table 3: Chemical Composition of Fermented Waste Agricultural residues ++

Substrates	DM	CP	CF	EE	ADF	LIGNIN
Rice husk	91.20	14.20[a]	52.75	10.50[a]	49.70[a]	42.60[a]
% Loss of original sample	3.36	-------	12.08	-------	17.71	24.20
% Gain of original sample	-------	24.78	-------	50.00	-------	-------
Cassava Waste	90.56	8.60[b]	45.00	10.53[a]	58.30[b]	39.50[a]
% Loss of original sample	-------	-------	48.57	-------	30.76	24.62
%Gain of original sample	2.05	164.61	-------	106.00	-------	-------
Groundnut shell	95.30	10.30[c]	30.21	2.50[b]	46.15[a]	41.50[a]
% Loss of original sample	-------	-------	19.44	-------	13.41	7.78
% Gain of original sample	1.65	53.75	-------	177.78	-------	-------
Cowpea shell	91.75	10.60[c]	42.50	5.90[c]	56.60[b]	47.23[b]
% Loss of original sample	------	-------	22.72	-------	33.02	23.20
% Gain of original sample	1.94	17.78	------	68.57	-------	-------
± SEM	10.20	2.35	5.18	3.28	4.80	2.50

P>0.05
++ completely randomized design model

Aspergillus niger improved the ash content increasingly post inoculation which was in agreement with previous studies (Jacqueline *et al.*, 1996). The crude fibre and other fibre fractions of the fermented substrates decreased significantly due to probably to the action of the fungus. This also agrees with the report of Rolz et al (1986) who found that fungus has

the enzymatic capacity of utilizing the hemicellulose content as a source of carbon and energy.

The values of crude fibre, acid detergent fibre (ADF) and lignin ranged from 30- 52.5%, 46.30 – 56.6% and 39.50 – 47.50% fermented substrates. The better performance of *Aspergillus niger* reported here supported the results of Abu et al. (1999). The levels of lignin and acid detergent fibre were higher in cowpea husk than rice husk.

There were significant differences in the contents of crude protein, crude fibre, ether extract and other fibre fractions (ADF, lignin) when the fermented products were subjected to ANOVA. However, highest crude protein and ether extract were recorded for rice husk followed closely by cowpea husk while cassava waste and groundnut shell were similar ($P > 0.05$). The high crude protein content and decreasing crude fibre and fibre fractions will likely enhance the use of *Aspergillus niger* as a biological means of fermenting some waste agricultural residues (Cassava waste, rice husk, groundnut shell and cowpea husk) for viable livestock feed and could also provides an alternative source of feed in ruminant nutrition.

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