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Nutrient enrichment of waste agricultural residues after solid state fermentation using *Rhizopus oligosporus*

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ABSTRACT

Objective: To study biodelignification of waste agricultural residues for the purpose of developing novel livestock feeds and feedstuffs.

Methodology and results: Waste agricultural residues of grain crops (maize cob, maize sheath, corn bran, sorghum stover, rice husk), root crop (cassava waste), leguminous crop (groundnut shell), Mansonia saw dust, Guinea grass and Feather meal were incubated with *Rhizopus oligosporus* in solid state fermentation,with untreated samples used as the control. Proximate analysis, mineral composition, fibre fractions and the invitro enzymatic digestibility of dry matter were determined and compared. The fungus was able to delignify the waste agricultural residues with significant ($P<0.05$) reduction in the crude fibre and lignin contents. Protein content of fermented waste agricultural residues increased significantly ($P<0.05$) above the unfermented samples and protein enrichment was highest with feather meal, followed by rice husk and was lowest for maize sheath. After incubation, maize cob produced a solid residue with a higher in-vitro dry matter enzymatic digestibility than the untreated samples.

Conclusion and application of findings: The study demonstrated that solid state fermentation of some waste agricultural residues with *Rhizopus oligosporus* can increase the level of limiting nutrients, e.g. proteins and minerals for monogastric and ruminant animals

Key words: *Rhizopus oligosporus*, residues, solid state fermentation, chemical composition.

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INTRODUCTION

There are various types of agricultural residues that are not useful to man and thus constitute a nuisance to the environment. About 147.2 million metric tons of fiber sources are found in the world (USDA, 1997), while the global output of wheat straw residue and rice straw were estimated at 709.2 and 673.3 million metric tons, respectively, in the 1990s (Mantanis, 1999). Additionally, the total global output of non-wood fibers was put at about 2.5 million metric tons (Atchison, 1995). About 61 million metric tons of crop residues are available in Nigeria (Stundstol & Owen, 1984). Out of these only 21% are consumed by sheep and goats and only if they are processed into acceptable and digestible forms (Stundstol & Owen, 1984).

Poor nutritive value of cereal crop residues with low digestibility, low crude protein and low mineral content has been reported to be a major constraint. However, these waste agricultural residues have some nutrients that are enveloped by lignin. Lignin, which is a part of the fibre

fraction, is highly resistant to chemical and enzymatic degradation while rumen microbes poorly degrade it. Chemical treatment of fibrous materials to break the ligninocellulose complex and make it more digestible is well documented (Belewu *et al*., 2004). However, some of the chemicals are poisonous and expensive while sodium load is increased in animals fed on diet with sodium hydroxide (Arndl *et al*., 1980). It has also been found that chemicals usually break the bonds between lignin and hemicellulose, and do not actually remove the lignin component (Belewu *et al*., 2004).

The current concepts of biological treatment in the degradation of fibrous materials are not adequately elucidated. Some authors have reported the utilization of fungi in the treatment of some fibrous materials (Belewu, 1999; Belewu & Banjo, 1999; Belewu, 2001; Belewu & Ademilola, 2002; Belewu *et al*., 2006). *Rhizopus oligosporus*, which belongs to the family mucoraceae, is widely used as a starter culture for the home based production of Tempeh (a soybean cake fermented with *Rhizopus* culture and incubated for a day or two and later cooked, popular in Indonesia as a protein source) (*Miszkiewcs et al., 2004*). The fungus produces various enzymes that hydrolyze the raw materials and change their texture, taste and aroma. The process also reduces or eliminates the anti-nutritive components in the fermented products. Enzymes secreted by the fungus could hydrolyse lipids, polysacchrides and protein (Nout & Rombouts, 1990). The thrust of this study was to evaluate the efficacy of *Rhizopus oligosporus* on delignification and saccharification of waste agricultural residues during solid state fermentation.

MATERIALS AND METHODS

Test organism: The *Rhizopus oligosporus* strain was obtained from the culture collections of the Department of Microbiology, University of Ibadan, Nigeria. Prior to the inoculation of the substrate, the fungus was maintained on potato dextrose agar (PDA) medium in Petri dishes. The fungus grew at 27°C and cultures were stored at 4°C.

Substrates: The substrates used included maize cob, cassava waste, sorghum stover, maize sheath, mansonia saw dust, rice husk, feather, corn bran, groundnut shell and guinea grass. All samples were autoclaved at 121°C (15psi) for 2 hours. After cooling each sample was divided into two equal parts; one part was inoculated with *Rhizopus oligosporus* and the other kept uninoculated to serve as control.

Inoculation and incubation: Fungal spores were harvested by flooding the Petri dish with Tween 80 solution and scrapping the mycelial surface to dislodge spores. The suspension was adjusted to 10^7 – 10^8 spores ml^{-1} using distilled water. One part of each of the substrate was inoculated with 5ml of the spore suspension. Inoculated substrates were incubated at ambient temperature. After an average of 7 days, the fungus had grown and covered all of the substrate. The substrates were later oven dried at 70°C for 48 h, before milling (using blender) and packing in labeled polythene bags.

Chemical analysis: Both fermented and control samples were subjected to proximate analysis according to standard procedures (AOAC, 1990). The fibre fraction was evaluated by the method of VanSoest (1963), while mineral analyses were done using atomic absorption emission spectrophotometer (AAS) model 200A. The phosphorus content was determined using Corning colorimeter model 253.

In vitro dry matter enzymatic digestibility: In vitro dry matter enzymatic digestibility (IVDMED) was determined by the method of Downman and Collins (1982). Each substrate (200mg) was suspended in 200ml solution of a 2g 1:1 pepsin in HCl (pH 4.8) and incubated at 40 °C for 48 h. Samples were centrifuged, washed once with distilled water, centrifuged and suspended in 200ml of a 2.5% (w/v) solution of the fungal, *Rhizopus oligosporus* and incubated again at 40°C for 48 h. The suspension was filtered, washed with distilled water and weighed. The controls had only distilled water (no fungus). During incubation the tubes were agitated twice daily. The IVDMED values represent percent weight loss due to enzymatic action in terms of initial dry matter of the samples, taking into account the weight loss of the control samples.

Data analysis: All data were subjected to the parametric student "T" test model (Snedecor & Cochran, 1971).

RESULTS AND DISCUSSION

The crude protein content increased in the fungus treated samples (Tables 1 & 2). The improvement in the crude protein content, which is nearly twice as high as in the beginning of the fermentation falls between 0.44 and 7.22% in cassava waste and feather meal, respectively. The increase may be due to the addition of fungal protein to the treated substrates during the fermentation process. This confirms the finding of Addae-Kagyah (1997); Belewu (1999), Belewu and Banjo (1999), Belewu (2001), Belewu (2003) and Belewu (2006). Additionally, Miszkiewicz *et al*. (2004) observed a similar increase in soluble protein concentration when *Rhizopus oligosporus* was used in the fermentation of pea seed.

Table 1: Chemical composition *of Rhizopus* fermented and unfermented maize cob, cassava waste, sorghum stover, maize sheath and sawdust.

Parameter (%)	Maize Cob (Untreated)	Maize Cob (Treated)	Cassava Waste (Untreated)	Cassava Waste (trted)	Sorghum Stovers (Untreated)	Sorghum Stovers (Treated)	Maize Sheath (Untreated)	Maize Sheath (Treated)	Sawdust (Untreated)	Sawdust (Treated)
Dry matter	92.00	97.40*	85.5	91.85*	90.40	92.30	91.06	94.70	93.42	91.75
Crude protein	3.28	3.94	5.47	5.91	3.94	4.60	2.19	3.28	2.84	3.94
Crude fibre	37.10	30.00*	18.40	10.30*	28.00	27.00	33.00	22.1*	68.60	54.8*
Ether extract	3.20	1.80	3.50	2.20	2.80	1.10	7.10	3.90	5.10	2.80
Ash	1.10	2.10	14.85	19.10	5.05	7.02	43.71	42.56	0.70	1.70
Lignin	33.20	31.38	71.50	63.75*	47.40	49.90	35.0	33.80	42.84	35.88*
NDF	83.70	83.43	85.20	82.45	68.65	65.78	70.56	68.20	80.49	74.86*
ADF	55.46	57.73	77.72	57.4*	46.25	45.58	43.71	42.56	68.20	63.90
Cellulose	50.50	52.05	13.70	18.7*	21.25	20.88	35.56	34.4	37.65	38.98
Hemicellulose	28.24	25.70	7.48	25.05*	22.40	20.20	26.85	25.64	12.29	10.96
Phosphorus	0.49	0.62	0.33	0.66	0.25	0.45	0.58	0.81	0.85	0.12
Magnesium	4.85	4.96	5.91	5.82	6.01	5.20	5.62	5.75	5.50	5.08
Sodium	4.69	4.47	4.62	4.65	4.60	5.01	4.63	4.92	4.84	4.87
Calcium	0.71	0.17	4.25	5.16	0.35	0.65	0.70	0.88	2.17	3.77
Iron (ppm)	42.58	1.22*	220.19	91.24*	24.33	1.22*	85.16	69.34*	138.68	55.96*
IVDMED	25.0	15.0*	45.0	45.0	35.0	45.0*	25.0	75.0*	20.0	25.0*

NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber; * indicates significant difference (P<0.05) between the treated and untreated types of each substrate.

The crude fibre content of the fungus treated samples decreased significantly (P<0.05) between 1.3 and 15% depending on type of residue (Tables 1 & 2). The reduction in the crude fibre content could probably be due to the action of the enzymes secreted by the fungus, as suggested by Miszkiewics *et al*., (2004). The lignin content and other fibre fractions (ADF, NDF and Cellulose) followed a similar trend as the crude fibre content (Table 1 and 2). The reduction in lignin content and other fibre fractions was similar to that reported by Rolz *et al*. (1986) indicating that fungi have the enzymatic potential to use lignocellulose component as sources of carbon and energy. This results in total biomass breakdown and lignin removal, accompanied by the removal of polysaccharides.

Based on the results the *Rhizopus oligosporus* strain used appeared to be lipolytic rather than lipongenic because the ether extract was significantly reduced after the fermentation process. The mineral composition of the fermented substrates was investigated because such information is needed for animal nutrition purposes. An interesting consequence of the fermentation was an increase in the mineral contents of the fermented products, though the magnesium content decreased slightly. The higher mineral content of the treated samples conforms to the report of Jacqueline *et al*. (1996).

Rhizopus oligosporus significantly increased the IVDMED of the solid residues of the various treated substrates as compared to the control suggesting that the fungus had modified the substrates making them more susceptible to enzymatic hydrolysis. Therefore, the increase in IVDMED values may clearly involve structural polymer modification and delignification.

The study showed that solid state fungal fermentation of some waste agricultural residues enhances the biomass protein and mineral contents and IVDMED, while the fibre fractions can be significantly reduced. The compositional improvement obtained is dependent on the enzymatic activities of the tested fungus hence fermentation of waste agricultural residues with *Rhizopus oligosporus* could help in production of novel feedstuff without compromising the quality for livestock production.

Table 2: Chemical composition *of Rhizopus* fermented and unfermented feather meal, rice husks, corn bran, groundnut shell and guinea grass.

Parameter (%)	Untreated Feather meal	Treated Feather meal	Untreated Rice Husk	Treated Rice Husk	Untreated Corn Bran	Treated Corn Bran	Groundnut Shell (untreat)	Groundnut Shell (treated)	Guinea Grass (untrt)	Guinea Grass (Treated)
Dry matter	85.5	92.65	91.40	92.25	87.4	93.75*	94.20	94.45	91.15	91.25
Crude protein	65.41	72.63*	9.19	10.94	8.31	9.63	5.25	6.34	2.41	5.47
Crude fibre	4.50	3.20	42.00	34.50*	7.00	5.70	71.90	56.60*	31.80	26.70*
Ether extract	3.00	1.10	5.20	3.90	15.10	13.30	4.40	2.70	3.20	1.20
Ash	2.25	3.95	19.05	21.00	5.20	9.70	4.05	5.70	5.10	8.05
Lignin	19.70	18.25	38.78	34.48	7.60	6.90	44.85	40.86	5.87	5.30
NDF	49.45	48.05	72.17	73.10	15.70	15.05	77.60	75.80	63.75	62.04
ADF	17.15	16.74	57.50	54.05	6.00	6.02	52.65	45.35*	39.95	37.14
Cellulose	29.75	29.80	33.39	38.62*	8.10	8.15	32.75	34.94	57.88	56.74
Hemicellulose	32.3	31.31	14.67	19.05	9.70	9.03	24.95	30.45*	23.80	24.90
Phosphorus	0.32	0.46	0.29	0.43	0.28	0.38	0.28	0.47	0.25	0.49
Magnesium	4.52	4.96	6.22	6.09	6.47	6.20	5.87	5.71	6.48	5.98
Sodium	4.29	4.98	4.70	2.48	4.95	4.80	3.21	4.70	4.89	4.98
Calcium	0.52	0.80	0.54	5.66*	0.043	0.32	0.14	1.55	0.34	7.01
Iron (ppm)	53.53	271.28*	593.18	68.12*	94.89	71.77*	334.54	178.83*	260.33	125.3*
IVDMED	60.0	50.0*	5.0	50.0*	60.0	90.0*	35.0	20.0*	30.0	70.0*

NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber; * indicates significant difference ($P<0.05$) between the treated and untreated types of each substrate.

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