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Bioconversion Impact of *Pleurotus ostreatus* on the Value of Rice and Groundnut by-products as Feed Resources

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The livestock industry in many developing countries is challenged with high cost of quality feed ingredients. For countries with agro-based economies, agro-industrial by-products are generated in large quantities but under-utilized and treated as "wastes". However their potential use as low-cost animal feed materials is challenged with digestibility problems and low protein content. The present study sought to evaluate the potential of fungal biotechnological (bioconversion) pre-treatment for transforming two major agro-wastes, namely rice (*Oryza glaberrima*) straw and groundnut (*Arachis hypogaea*) shells, into valuable animal feeds. In a Completely Randomized Block Design, rice straw (RS), groundnut shells (GS) and a 1:1 ratio mixture of RS and GS were separately subjected to a 5-week *Pleurotus ostreatus* solid-state fermentation (SSF) process. The study demonstrated that *P. ostreatus* SSF increased ($P<0.05$) the level of limiting nutrients particularly, proteins and minerals (P, K and Ca) while at the same time decreasing the fibre and tannin levels to enhance their digestibility for monogastrics and ruminants.

Key words: Agro-byproducts, Solid-State Fermentation, *Pleurotus ostreatus*, Bioconversion

Several challenges confront the livestock industry in Ghana. Provision of adequate quantity of quality feed all year round is one such major challenge. In the poultry industry for instance, feeding alone accounts for about 60-80% of the production cost (Sindhu *et al.*, 2002; Tewe, 1997). Crop residues and other agricultural by-products, have become major components of livestock feed in many parts of the world (Jayasuriya, 1985). However, the use of many of these by-products as livestock feed materials is limited by high levels of some non-nutritive components (such as lignin and silica) as well as anti-nutritional factors (such as tannins), which interfere with the normal digestion, absorption and metabolism of the feed (Pathak, 1997; Wardlaw and Insel, 1995). Appropriate processing such as the application of bioconversion pre-treatment methods involving bacteria and fungi can improve their quality (such as increase in the protein content) and efficient livestock utilization of these agro-byproducts (Beguin, 1990). White-rot fungi such as *Pleurotus* species have been found to be very useful in

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recycling organic wastes as well as improving upon the nutritive values of various lignocellulosic agro-industrial byproducts (Alemawor *et al.*, 2009). The objective for this study was to evaluate the effect of *Pleurotus ostreatus* (oyster mushroom) fermentation on the proximate composition and the individual fibre components of the agro-industrial byproducts; rice (*Oryza glaberrima*) straw, groundnut (*Arachis hypogaea*) shells and their mixture.

MATERIALS AND METHODS

Sources of substrates

Rice straw was obtained from a farm at Tamale in the Northern region of Ghana. Groundnut shells were obtained from a local groundnut processing centre in Tamale market. *Pleurotus ostreatus* grain spawn was procured from ROB-ART Enterprise (a local mushroom-producing enterprise) at Kenyase, Kumasi. All relevant equipment as well as analytical reagents and chemicals were obtained from the laboratory of the Biochemistry and Biotechnology Department, KNUST, Ghana. Aluminium trays (15cm × 11cm × 4cm) were procured from the local market in Kumasi.

Substrate Processing and *P. ostreatus* solid-state treatment

The residues were manually cleaned and sorted (by hand-picking) to remove foreign matter. They were then solar-dried to a moisture content of 10%. Rice straw was chopped into lengths of 1-4 cm, while the groundnut shells were coarsely pounded (using mortar and pestle) to an average size of 0.6 cm^2. In total three different substrates were experimented under the same conditions:

i. Solid-State Fermentation (SSF) of rice straw (RS).
ii. SSF of groundnut shells (GS).
iii. SSF of mixture of RS and GS in the ratio of 1:1.

Fifteen kilograms (15 kg) of each substrate type were soaked with clean tap water in a concrete basin for 5 h. Soaking was done to ensure that the residues absorbed enough water as well as to wash off soil particles. The substrates were allowed to stand for 3 h after draining the water. Two hundred and fifty gram (250-g) portions of each of the moistened residues were weighed and transferred into the cleaned and labelled aluminium trays and the open end of the trays covered with polyethylene films and aluminium foil. The trays and their contents were then steam-pasteurized for 4 h in a 200-L metal barrel. After pasteurization, the trays were taken immediately to a previously disinfected inoculation room. There were 40 trays for each substrate: 20 served as control (i.e. pasteurized but not inoculated with *Pleurotus ostreatus*) and the 20 other were labelled as *Pleurotus ostreatus*-inoculated.

The pasteurized tray-packed substrates were allowed to cool to room temperature and then aseptically inoculated with the *P. ostreatus* spawn grains (1g per tray) using sterilized inoculation spoon. With the aid of a sterilized stainless pin, the introduced spawn grains were evenly distributed over the surface of the substrates, before quickly covering with the polyethylene film (perforated with few micro-holes) and the foil and subsequently holding firmly with rubber band and cellotape. The trays were then arranged on shelves in an incubation room for fermentation to commence.

Experimental design, Sampling and Chemical Analyses of Samples

The Completely Randomized Block Design (CRBD) was adopted for the study. Fermentation lasted for five weeks, and triplicate sampling per substrate treatment was carried out weekly for compositional analyses after solar-drying for 3 days. In addition, the composition of raw samples (i.e. not pasteurized and pasteurized but not

Pleurotus ostreatus-inoculated) per substrate was analyzed for their nutrient levels. Proximate analysis of the samples was conducted according to standard AOAC (1990) protocols. The fibre fractions were estimated by the Van Soest (1994) detergent procedure, while total phenolics and tannins of samples were determined using the Folin-Ciocalteu method as described by Makkar *et al.* (1993). Potassium content was determined using a flame photometer (JENWAY PFP 7, England) while phosphorus and calcium were measured by employing a spectro-photometer (OPTIMA SP-300, Japan).

Measurement of total sugar and in vitro dry matter digestibility (IVDMD)

A 1M $NaHPO_4$ buffer (150 mL; pH=6.8) and 1.3U.ml^{-1} α-amylase (0.4 mL) were added to sample (2 g) and the mixture incubated at a temperature range of 36-39 $^{\circ}$C for 4 h. At the end of the incubation period, 1% (w/v) NaOH (5 mL) was added to the samples and the suspensions filtered using a Whatman No. 1 filter paper. The amount of reducing sugars present in each filtrate was determined using a refractometer (ABBE 60, UK) and the sugar level recorded in degree brix. Controls were without the enzyme.

Analysis of data

Data (presented as mean of triplicate determinations ± standard deviation) were subjected to analysis of variance (ANOVA) using STATGRAPHICS® Centurion XV and the Duncan's multiple range test was carried out to detect significant differences (defined as $P < 0.05$) between treatment means.

RESULTS AND DISCUSSION

Plant biomass regarded as "wastes" are biodegradable and can be converted into valuable animal feeds (Howard *et al.,* 2003). The composition of crop residues varies with variety, location, and the cultural practices employed in growing the crop from which they are obtained. Table 1 shows the determined proximate composition and contents of some minerals for the substrates.

The crude protein of the *Pleurotus*-treated samples increased significantly ($P<0.05$) by 56-127 percent depending on type of residue (Table 1). The increase in crude protein may be due to the addition of fungal protein or the bioconversion of carbohydrates in the colonized substrates into mycelia protein or single cell protein (SCP) by the growing fungus during the fermentation process (Iyayi, 2004). It may also be partly due to the secretion of some extracellular enzymes such as cellulases and amylases by the fungus in an attempt to use cellulose and starch as sources of carbon (Raimbault, 1998; Oboh *et al*., 2002).

The crude fibre content of the *Pleurotus*-treated samples decreased significantly ($P>0.05$) between 38 and 42 percent depending on type of residue (Table 1). The reduction in the crude fibre content could probably be due to the activity of the enzymes secreted by the fungus, as observed by Miszkiewics *et al*. (2004). Rolz *et al*. (1986), Kutlu *et al*. (2000), and Alemawor (2009), also reported of similar reduction in crude fibre and fibre fractions (hemicellulose, cellulose and lignin) of the substrates they fermented with *P. ostreatus*. This is an indication that fungi have the enzymatic potential to use lignocellulose component as sources of carbon and energy. Following the fungal fermentation, a 29-64% reduction ($P<0.05$) in ether extract was observed in the substrates. Depending on the residue, the fermentation treatment showed significant ($P<0.05$) and variable increments of 72-89% in calcium, 56-141% in phosphorus and 60-73% in potassium (Table 1).

The observation of high mineral contents of the treated samples conforms to the reports of Jacqueline *et al.* (1996), Alemawor *et al.* (2009), and Belewu and

Babalola (2009). Minerals are required for tissue growth and the regulation of body functions. They are normally categorised as macro-minerals (when required in the order of g/day) or as micro-minerals (when required in mg/day or less). About 20-22 mineral elements have been shown to be essential to animal nutrition (Little, 1985). Animals experience symptoms of deficiency or toxicity if a particular mineral is missing in the diet or is in excess, respectively (Church and Pond, 1982).

Table 1: Chemical composition of *Pleurotus ostreatus* treated and untreated substrates

Parameter (%)	RS (untreated)	RS (treated)	GS (untreated)	GS (treated)	RSGS (untreated)	RSGS (treated)
Dry Matter	93.23±0.10	86.98±0.22	94.33±0.01	88.74±0.18	93.70±0.20	88.87±0.03
Crude Protein	2.85±0.44	6.46±0.10	5.32±0.25	8.80±0.22	5.03±0.44	7.85±0.23
Crude Fibre	33.35±0.06	20.32±0.03	65.46±0.57	37.80±0.10	49.01±0.22	29.56±0.22
Ether Extract	1.05±0.03	0.54±0.23	2.28±0.03	1.62±0.03	1.74±0.08	0.62±0.10
Ash	14.83±0.19	23.65±0.23	4.13±0.05	7.08±0.06	9.23±0.23	14.78±0.06
NFE[1]	47.93±0.45	49.14±0.06	22.87±0.71	44.71±0.20	34.98±0.67	47.19±0.18
NDF[2]	66.20±0.23	42.43±0.18	77.85±0.34	61.42±0.18	71.25±0.13	51.34±0.06
ADF[3]	48.10±0.18	31.75±0.06	60.00±0.18	47.26±0.06	54.89±0.20	38.51±0.23
Hemicellulose	18.09±0.06	10.68±0.23	17.86±0.19	14.16±0.18	16.30±0.25	12.83±0.20
Lignin	12.41±0.07	6.44±0.20	18.52±0.51	11.87±0.23	15.46±0.10	9.31±0.20
Cellulose	35.69±0.24	25.32±0.18	41.48±0.34	35.48±0.18	39.43±0.16	29.20±0.07
Potassium (mg/g)	0.29±0.20	0.50±0.07	0.10±0.24	0.16±0.18	0.15±0.18	0.26±0.06
Phosphorous (mg/g)	0.32±0.06	0.50±0.18	0.11±0.07	0.18±0.20	0.22±0.24	0.53±0.24
Calcium (mg/g)	0.70±0.06	1.32±0.18	0.22±0.06	0.39±0.18	0.46±0.07	0.84±0.18

[1]NFE=Nitrogen Free Extract; [2]NDF=Neutral Detergent Fiber; [3]ADF=Acid Detergent Fiber;
RS= rice straw; GS= groundnut shell; RSGS=50% rice straw + 50% groundnut shell

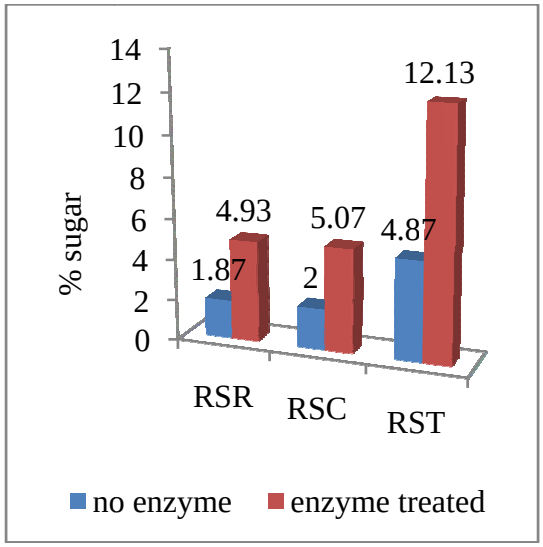

Figure 1: Changes in soluble **sugar content** following fermentation of RS with *P. ostreatus* (RSR-Raw rice straw, RSC-Control rice straw, RST- fermented/treated rice straw)

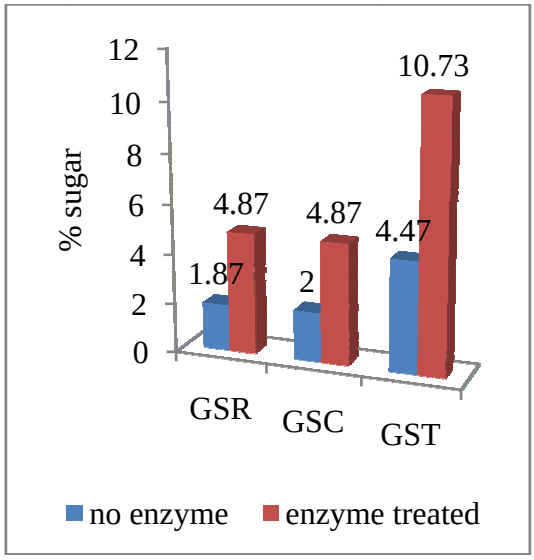

Figure 2: Changes in soluble **sugar content** following fermentation of GS with *P. ostreatus*

Pleurotus ostreatus significantly increased the IVDMD of the 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 (Figures 1-3). Therefore, the increase in IVDMD values suggests structural polymer modification and delignification as a result of the fungal fermentation.

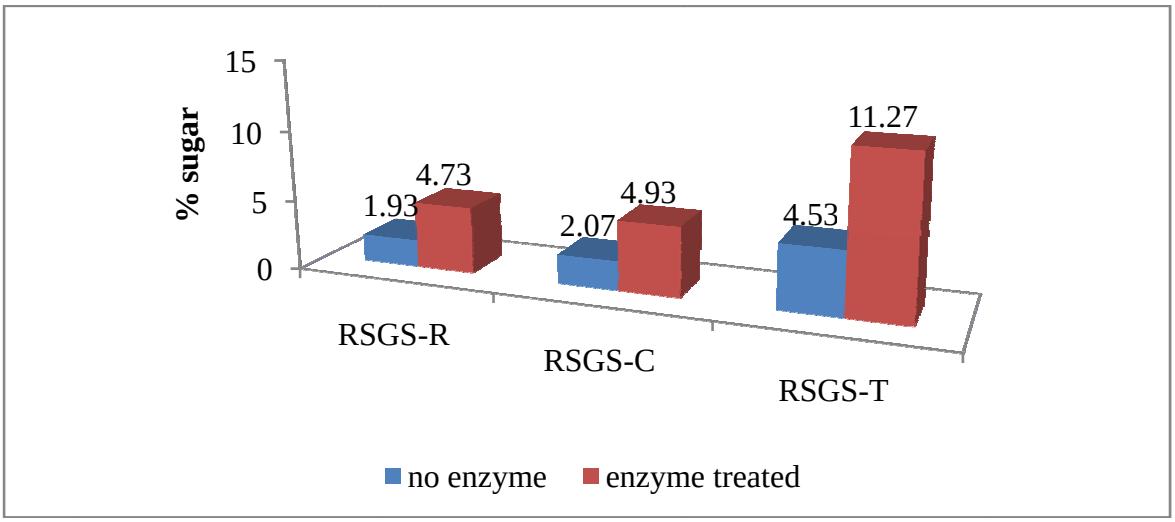

Figure 3: Changes in soluble **sugar content** following fermentation of RSGS with *P. ostreatus*

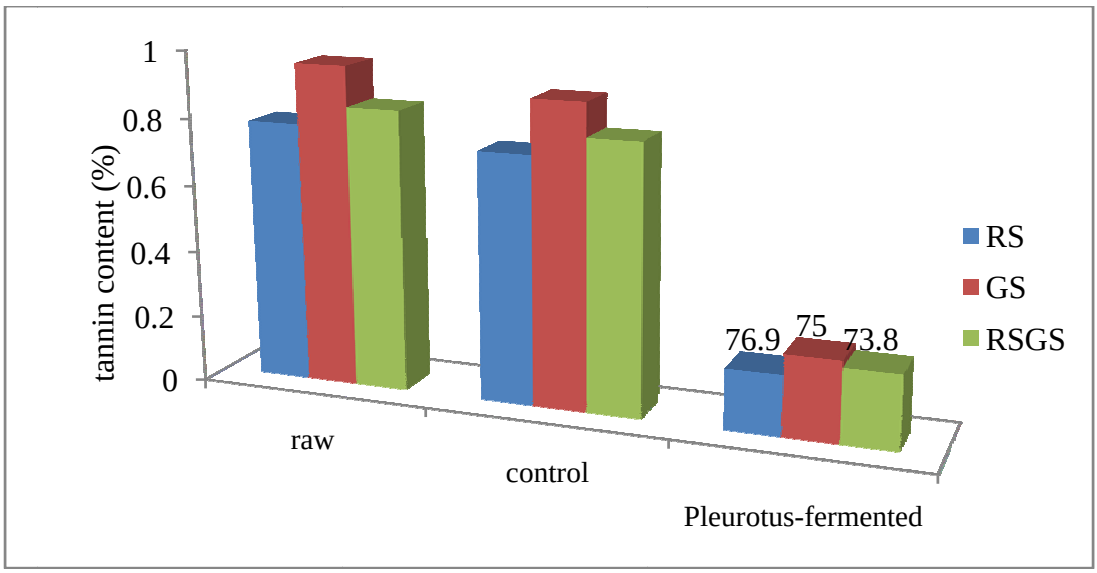

Figure 4: Changes in **tannin** content of substrates following fermentation with *P. ostreatus* (numbers on bars indicate % decrease over controls)

Tannin level for all the substrates decreased significantly (P>0.05) between 73 and 77 percent (compared to their respective controls) after their optimum fermentation with *P. ostreatus* (Figure 4). This agrees with observations reported by Alemawor *et al.* (2009). Therefore, by these results *P. ostreatus* fermentation enhances the feed value (more protein available) of the agro-residues as potential feed source.

CONCLUSION

The observed compositional improvement obtained (reduction in fibre fractions and enhancement in the biomass protein and mineral contents as well as IVDMD) in the agricultural residues suggests that the solid state treatment involving edible fungi can be a useful and a promising cost-effective technique for developing novel feedstuff from agro-wastes for livestock production.

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