

Full Length Research Paper

Effect of *Pleurotus ostreatus* fermentation on cocoa pod husk composition: Influence of fermentation period and Mn²⁺ supplementation on the fermentation process

Francis Alemawor^{1*}, Victoria P. Dzogbefia¹, Emmanuel O.K. Oddoye² and James H. Oldham¹

¹Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

²Cocoa Research Institute of Ghana, P.O. Box 8, New Tafo-Akim, Ghana.

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Cocoa pod husk (CPH) is a major agro-industrial residue in Ghana with a potential value as a low-cost unconventional feedstuff for livestock. However, its effective use is limited by poor nutrient composition, mainly due to its high lignocellulose or fibre and also low protein levels. White-rot fungi such as *Pleurotus* species are bio-catalytic systems for bioconversion processes such as the bioconversion of lignocellulose materials into value-added products including nutritious animal feed. Presence of metals such as manganese (II) ions is reported to enhance fungal enzyme activity in the bioconversion of industrial lignocellulosic residues. The current study investigated the viability of using and optimising a fermentation process involving edible oyster mushroom (*Pleurotus ostreatus*) as biocatalyst to improve the nutritional status of CPH. Fermentation period and level of manganese (Mn²⁺) ion supplementation of CPH were the two major factors of the fermentation process evaluated and optimised in this study. Mn supplementation was critical in producing a positive bioconversion effect on CPH by *P. ostreatus*. Five (5) weeks of *P. ostreatus* solid-state fermentation of CPH amended with MnCl₂ at 0.075% (w/w) concentration, was observed as an economic and optimum treatment to produce positive and significant (P < 0.05) changes in CPH composition, i.e. 36% increment in crude protein and total soluble carbohydrates; 17% reduction in crude fibre and lignin as well as 88% reduction in total tannins.

Key words: Agro-industrial by-product, bioconversion, cocoa pod husk, manganese (II) ions, non-starch polysaccharides, *Pleurotus ostreatus* solid-state fermentation.

INTRODUCTION

The fruit of the tropical plant, cocoa (*Theobroma cacao* L.) is an important commodity in Ghana because of the economic value of its seeds or beans. Cocoa pod husk (CPH), which forms over 70% (w/w) of the whole matured cocoa fruit, is a major by-product from the cocoa industry but is currently under-utilised. Thus at every cocoa harvesting season, enormous quantities of CPH become

available but discarded as 'waste.' Ghana is currently the world's second largest producer of cocoa after Côte d'Ivoire, with annual production level of over 600,000 metric tonnes (ICCO, 2003). Previous works revealed the potential use of CPH as an unconventional low-cost feed ingredient for livestock nutrition; possibly reducing feed costs by replacing some of the expensive conventional feed ingredients used in ration formulation (Atuahene et al., 1984; Sobamiwa, 1998). However, the replacement value for CPH in diets is limited by its poor nutrient composition. Its low protein value coupled with the presence of high amounts of lignin (14% w/w) as well as non-starch polysaccharides (NSPs) including hemicellulose (11% w/w) and cellulose (35% w/w), which are poorly utilised by farm animals (particularly monogas-

*Corresponding author. E-mail: alefracid2000@yahoo.com. Tel: +233-20-8780625. Fax: +233-51-60137.

Abbreviations: CPH, Cocoa pod husk; **CU-S**, control substrate without Mn amendment; **Mn-S**, Mn-amended substrate; **TSCHO**, total soluble carbohydrates or total sugars.

trics) constitute major limitation to its replacement value in animal diets (Annison, 1990; Sobamiwa, 1993).

Application of effective bio-treatment approaches such as fungal biotechnology is worth considering for the improvement of the nutritional value of CPH. Higher fungi or mushrooms have the ability to bio-transform fibrous agro-residues into value-added products through their extracellular enzyme activities (Wood and Fermor, 1982). Aside, culture conditions such as suitable moisture and temperature to support fungal growth, metal ions such as manganese (Mn) have been reported to influence or improve white-rot fungi bioconversion activity in the biodegradation of lignocellulose in agro-industrial by-products (Kerem and Hadar, 1995; Wuyep et al., 2003). This study was carried out to assess the effect of a solid-state fermentation treatment involving the edible fungus, *Pleurotus ostreatus*, on the composition of CPH. In addition, the influence of Mn(II) ion amendment and fermentation period on the process was evaluated.

MATERIALS AND METHODS

Preparation of CPH and *Pleurotus* culture

Fresh cocoa pod husks were obtained and processed at Cocoa Research Institute of Ghana (CRIG), New Tafo-Akim. The husks were cleaned, chopped into smaller pieces (size of ≤ 3 mm), pelleted (size of 5-10 mm) and solar-dried to a moisture content of ca. 10%. The dried husk pellets were then stored in polythene sacks until needed for the study. Fresh *P. ostreatus* grain spawn was procured from a local mushroom producer at Kenyase, a suburb in the Kumasi Metropolis of Ghana.

P. ostreatus solid-state fermentation of CPH

A 10-kg portion of dried CPH pellets was mixed thoroughly with clean tap water to adjust the moisture content to about 65% and labelled as Control Substrate (CU-S). To a second 10-kg lot of dried CPH pellets, clean tap water plus manganese (Mn) ions in the form of MnCl_2 (at 1% w/w or 10000 ppm) was added and then mixed thoroughly to obtain a mixture with moisture content adjusted to about 65% and labelled as Mn-amended Substrate (Mn-S). The two formulated substrates were then transferred into separate clean plastic containers, covered and made to stand at room temperature for 3 days to ensure effective water adsorption onto the CPH material as well as to stabilize the medium. One and a half (1.5) kg portions of each substrate were subsequently transferred into labelled aluminium (Al) trays (30 cm x 22 cm x 4 cm) with 24 trays per substrate, and the open ends of the trays were covered with polyethylene films and Al foil. The trays and their contents were then steam-pasteurized for 2 h in a 200-L metal barrel. After cooling to room temperature, the pasteurised substrates were inoculated aseptically with the *Pleurotus ostreatus* grain spawn and incubated in a dark room at 23-25°C and approximately 80% relative humidity. During the incubation, 2 out of the 24 trays (containing the spawned substrate) per substrate type were randomly sampled at the end of each week and the contents were solar-dried to an average moisture content of 9%. The *Pleurotus* fermentation process and sampling were terminated after seven (7) weeks due to cessation of mycelial growth of *P. ostreatus* and subsequent contamination of the substrates by foreign moulds.

Chemical analyses

The solar-dried samples were then prepared (crushed with pestle and mortar to a particle size of ≤ 1 mm) for compositional analyses. Standard methods according to AOAC (1990) were used for determination of ash (method 942.05), protein (method 954.01) and fibre (method 962.09). Measurement of total sugars (TSCHO) was done using the anthrone- H_2SO_4 reaction method as described in the ILRI (1997). In addition, the levels of lignin-sa (determined by solubilization of cellulose with sulphuric acid), aNDF (assayed with a heat stable amylase and expressed inclusive of residual ash), and ADF (expressed inclusive of residual ash fractions) were determined according to procedures described by Robertson and Van Soest (1981), Mertens (2002) and AOAC (1990)—method 973.18, respectively. The values of these fibre fractions were subsequently used to estimate levels of hemicellulose (i.e. difference between NDF and ADF values) and cellulose (i.e. difference between ADF and lignin-sa values) in the samples. The Folin-Ciocalteu method according to Makkar et al. (1993) was employed to determine levels phenolics and tannins in CPH before and after optimum *Pleurotus*-fermentation. Mean values for all determinations were recorded on dry matter basis.

Experimental design and data analysis

A factorial design was adopted, with two (2) treatments of the CPH substrate (i.e. CU-S and Mn-S) and seven (7) fermentation periods (i.e. 1, 2, 3, 4, 5, 6, 7 weeks) as well as two (2) replications. Analysis of variance calculations and comparisons were conducted on data obtained, using GENSTAT Release 7.2 DE program for PC/Windows XP (2007). Where the analysis of variance revealed the existence of significant differences among the treatment means at the 5% level, the Fisher's (*protected*) Least Significant Difference (LSD) was used to locate treatment means that were significantly different from one another.

RESULTS AND DISCUSSION

Effect of fermentation time and manganese amendment on *Pleurotus* treatment of CPH

The influence of manganese amendment and fermentation period on the bioconversion of CPH by *P. ostreatus* was evaluated by monitoring the compositional changes in the CPH material during the fermentation process. Following the fungal treatment, proximate composition of the CPH changed with fermentation period (Figure 1). The ash content for each substrate increased ($P < 0.05$) with fermentation period and ranged between 91.0–134.0 g/kg. The observed increase in ash was probably due to decline in some organic fractions of CPH during the bioconversion process (Zadrazil et al., 1995; Jalč et al., 1999).

Crude protein content of the substrates increased significantly ($P < 0.05$) and correlated positively ($r = 0.9684$ for CU-S; $r = 0.963$ for Mn-S) with fermentation period (Figure 2). At the end of 6 weeks of fermentation, 32.28 and 39.93% peak increases in protein were obtained for CU-S and Mn-S, respectively; although the percent gains measured at the end of weeks 5, 6 and 7 were similar for both substrate types. The manganese pretreatment resulted in larger increases in CPH protein

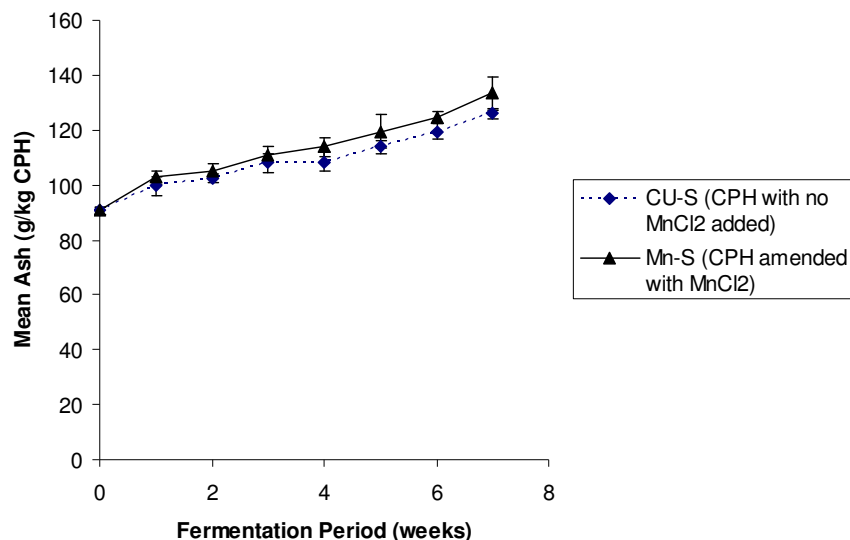


Figure 1. Changes in total ash during *Pleurotus* solid-state fermentation of CPH formulated substrates.

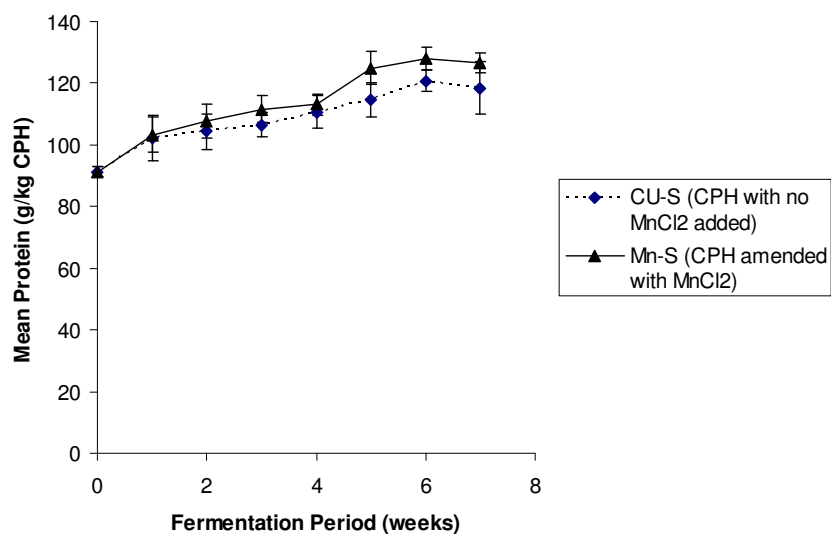


Figure 2. Changes in crude protein during *Pleurotus* solid-state fermentation of CPH formulated substrates.

level than the control. Previous studies on fungal fermentations of agro-residues – including cassava by-products, wheat straw, coffee husk, corn bran and rice bran – have also reported similar increases in protein content (Balagopalan, 1996; Leifa et al., 2001; Iyayi and Aderolu, 2004). A recent solid-state fermentation study, involving a newly isolated fungal strain *Rhizopus stolonifer* LAU 07, however quoted ca. 95% increase in CPH protein (Lateef et al., 2008). The observed increase in protein level in this work could be due to bioconversion of some of the soluble carbohydrates in the colonised substrate into mycelia protein or single cell protein (SCP) by the growing fungus (Iyayi, 2004). Higher fungi or mushrooms have been reported to be capable of transforming

nutritionally low-grade agro-wastes into protein-rich bio-products. Mycelia proteins of edible fungi are of high biological value as they are rich in essential amino acids (Alofe et al., 1998; Manzi et al., 1999). Most of the extracellular fungal enzymes produced by the growing fungus during bioconversion process are proteinaceous in nature; thus spent enzymes could contribute some amount of protein to the substratum (Kadari, 1999). Generally the observed increase in the protein content indicates a positive bioconversion effect of *P. ostreatus* on CPH.

Crude fibre concentration of the substrates decreased significantly ($P < 0.05$) and inversely ($r = 0.9668$ for CU-S; $r = 0.9525$ for Mn-S) related with fermentation period

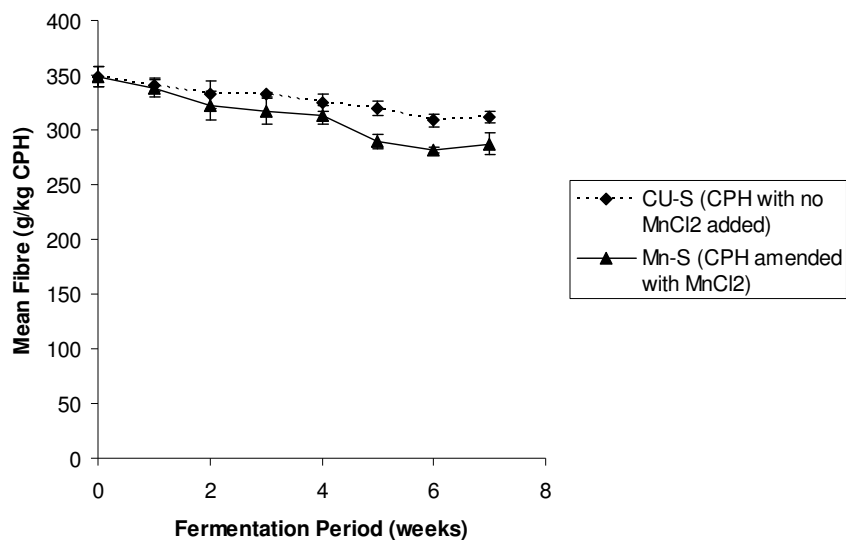


Figure 3. Changes in crude fibre during *Pleurotus* solid-state fermentation of CPH formulated substrates

(Figure 3). Average reductions of 8.28 and 17.08% were obtained in the control non-amended and Mn-amended substrates respectively, by the end of 5 weeks of fermentation with no significant changes thereafter. Greater fibre degradation was observed in the Mn-amended substrate than the control non-amended especially at the 5th, 6th and 7th weeks (Figure 3). Kutlu et al. (2000) have also reported reduction in crude fibre level of wheat straw following incubation with *Pleurotus florida*, while Lateef et al. (2008) reported a 7.2% decrease in CPH crude fibre content. The decline in fibre content is an indication of *P. ostreatus* having enzymatic system for degradation of polymeric lignocellulose of CPH. Ability of fungi to degrade fibre in agro-residues including cassava peels, cereal bran, palm kernel meal and coffee by-products has been previously reported (Leifa et al., 2001; Iyayi and Aderolu, 2004). Thus the observed results are consistent with previous reports by other workers.

Changes in lignocellulose composition of CPH following *Pleurotus* treatment

Lignin, cellulose and hemicellulose fractions form the bulk of CPH fibre. During the fermentation process, the weekly changes observed in the levels of these fibre fractions indicated the degree of lignocellulose biodegradation as well as the probable enzyme activities exhibited by *P. ostreatus* on CPH. Direct assays for activities of enzyme types detected were however not carried out in this study due primarily to logistic constraints.

Hemicellulose content of the CPH substrates decreased significantly ($P < 0.05$) and inversely ($r = 0.867$ for CU-S; $r = 0.9486$ for Mn-S) related with fermentation period (Figure 4). A 20.52% maximum reduction was observed at end of the 5 weeks of fermentation of CU-S,

while the Mn-S showed a 22.78% peak reduction at the end of 7 weeks. However, the reductions in hemicellulose content observed at the end of 5, 6 and 7 weeks of fermentation compared favourably ($P > 0.05$) for each substrate type (Figure 4). Decrease in hemicellulose content with time may be an indication of the presence of hemicellulolytic enzyme activity by *P. ostreatus* on CPH. *Pleurotus* species can produce enzymes that will hydrolyse a variety of β -(1, 4) linked glucan substrates as well as various glycosides (Highley, 1976). Significant hemicellulolytic activities by *Pleurotus* spp. on lignocellulosic materials have also been reported (Buswell et al., 1993). Though the results obtained generally showed higher rates of hemicellulose degradation for the Mn-amended substrate compared to the control non-amended type, the difference between the two substrates on weekly comparison basis was insignificant ($P > 0.05$). This suggests that the *Pleurotus* bioconversion activity on CPH hemicellulose was marginally influenced following the Mn-amendment which implies that Mn ion may not have any major role in the CPH hemicellulose degradation.

Pleurotus degradation of CPH cellulose followed a trend similar to that observed for the hemicellulose fraction. As fermentation period increased, cellulose content decreased significantly ($P < 0.05$) with the control non-amended and Mn-amended substrate types showing peak reductions of 18.20 and 26.34% at the end of 7 and 6 weeks, respectively (Figure 5). However, the reductions in cellulose fraction observed at the end of 5, 6 and 7 weeks of fermentation were similar ($P > 0.05$) for each substrate type. Aracelly-Vega et al. (2005) also reported reduction in cellulose of coffee pulp and banana leaves after *P. ostreatus* cultivation. In an earlier work, Iyayi (2004) also observed reduction in cellulose following *Aspergillus* fermentation of agro-residues including wheat

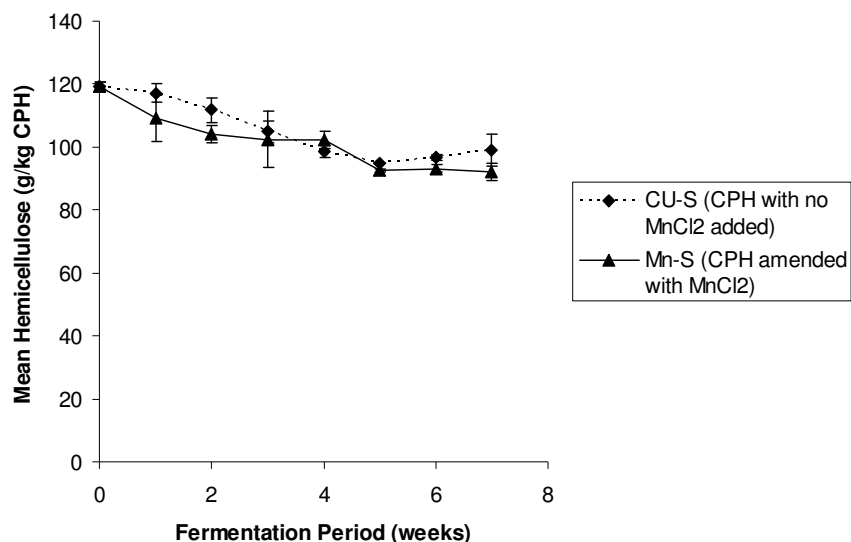


Figure 4. Changes in hemicellulose during *Pleurotus* solid-state fermentation of CPH formulated substrates.

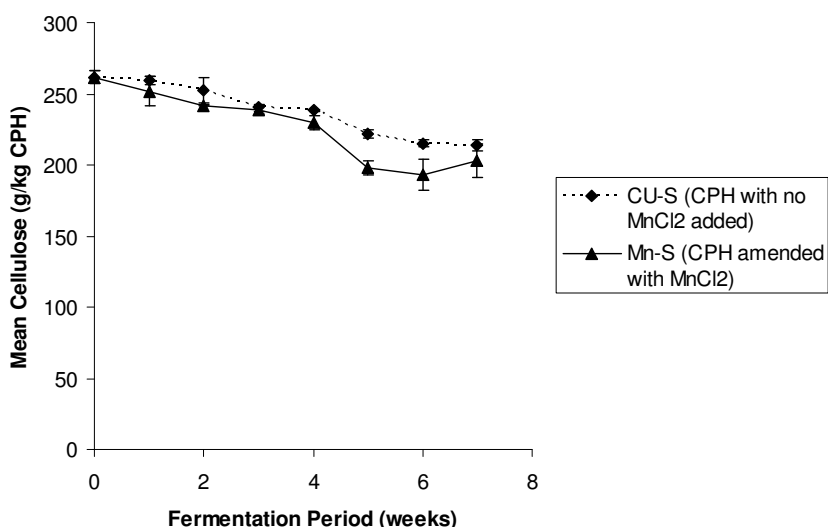


Figure 5. Changes in cellulose during *Pleurotus* solid-state fermentation of CPH formulated substrates.

offal, brewers dried grains and maize offal. A concert action of extracellular fungal hydrolases, collectively called cellulases enables fungi to degrade cellulose material (Datta and Chakravarty, 2001). The current results also showed that the Mn-amendment of CPH was advantageous as *Pleurotus* cellulose degradation was enhanced by the 5th week (Figure 5). Baldrian et al. (2005) observed that cellulolytic and hemicellulolytic activities of *P. ostreatus* on wheat straw were less prone to metal ion effect of Mn ($P > 0.05$). However, an earlier *Pleurotus* solid state fermentation study by Kerem and Hadar (1995) reported approximately 25% increase in the rate of cellulose breakdown in cotton stalks amended with Mn^{2+} ions; though this was concentration dependent.

Our results are thus consistent with those of Kerem and Hadar (1995). Perhaps the Mn ion effect could be substrate specific.

Lignin concentration of CPH decreased significantly ($P < 0.05$) and correlated inversely ($r = 0.8147$ for CU-S; $r = 0.9938$ for Mn-S) with fermentation period (Figure 6). The results showed a pronounced ($P < 0.05$) delignification of Mn-amended substrate resulting in 17.06, 21.69 and 23.54% decline in lignin fraction following 5, 6 and 7 weeks of *Pleurotus* fermentation, respectively (Figure 6). In contrast, only a maximum of 3.03% decrease in lignin was observed in the control substrate after 6 weeks fermentation (Figure 6). In the biodegradation of lignin, white-rot fungi produce extracellular enzymes including

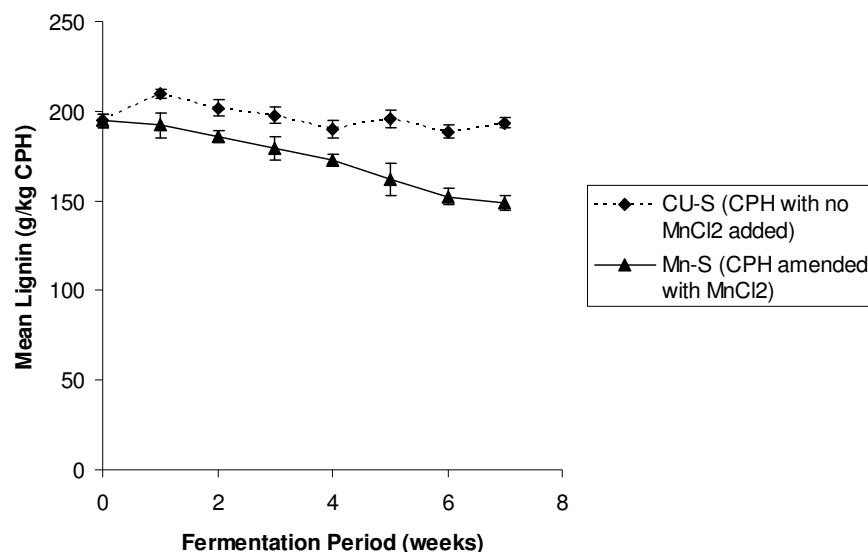


Figure 6. Changes in lignin during *Pleurotus* solid-state fermentation of CPH formulated substrates.

laccases and peroxidases that oxidize both the aromatic rings and aliphatic side chains to produce low-molecular weight products (Lo et al., 2001). Ortega et al. (1992) observed that *Pleurotus* species exhibit strong ligninolytic activity on lignocellulosic residues including sugarcane crop residues. The significantly lower lignin values observed for Mn-amended compared to that of the control substrate indicates that Mn^{2+} ion enhances *Pleurotus* bio-delignification. Manganese (Mn^{2+}) is a crucial substrate for manganese peroxidases (MnPs) – the most abundant group of extracellular ligninolytic enzymes in white-rot fungi (Hatakka, 1994). MnP oxidizes Mn^{2+} to highly reactive Mn^{3+} which in turn oxidizes the phenolic moieties in lignin to unstable free radicals (phenoxy radicals) and decomposition spontaneously occurs (Hofrichter, 2002). In a solid-state fermentation of wheat straw, Baldrian et al. (2005) reported an increase in the activity of the ligninolytic system (particularly laccase) of *P. ostreatus* (resulting in an enhanced delignification rate) when wheat straw was supplemented with Mn ions. Additionally, a previous work reported *P. ostreatus* degrading 50–56% of lignin in cotton stalks amended with Mn at <1% w/w (Kerem and Hadar, 1995). Thus the nature of the substrate, the concentration and bio-availability of Mn present in the substrate; and fermentation period are potential factors that may influence the extent of delignification by *Pleurotus*.

Apart from the observations that the Mn-amendment promoted ($P < 0.05$) delignification of CPH than the control non-amended, and that lignin level of CPH decreased ($P < 0.05$) with fermentation period, the analysis of variance (ANOVA) results further showed evidence ($P < 0.05$) of interaction between the two main variables – substrate treatment (i.e. non-amended and Mn-amended) and length of fermentation period. This implies that the

effect of substrate treatment on lignin degradation by *Pleurotus* depends on the length of fermentation period. *Post hoc* one-way ANOVAs to examine the simple effects of the observed interaction showed that delignification (measured by the decrease in lignin concentration of CPH) was insignificant ($P > 0.05$) after 3 and 5 weeks for the control non-amended and Mn-amended substrates, respectively.

Changes in total soluble carbohydrates of CPH following *Pleurotus* treatment

Generally, the total soluble carbohydrate (TSCHO) of CPH increased ($P < 0.05$) and correlated directly ($r = 0.8146$ for CU-S; $r = 0.7089$ for Mn-S) with fermentation period (Figure 7). An initial decrease in total soluble carbohydrates observed in the 1st week of fermentation was followed by significant weekly increases ($P < 0.05$) which peaked at the end of 6 weeks of fermentation, giving 16.72 and 42.25%, respectively, for the control and Mn-amended substrates. Hydrolysis of CPH polysaccharides, including hemicellulose and cellulose, primarily due to the extracellular enzyme activity of *P. ostreatus* may have contributed to the increase in soluble carbohydrates from 2 – 6 weeks of fermentation (Figure 7). The later decline observed after 6 weeks of fermentation may be attributed to a decrease in enzyme activity on the CPH polysaccharides.

Iyayi and Aderolu (2004) reported similar increase in soluble sugars of agro-industrial by-products including brewers dry grains, palm kernel meal and cereal bran after solid state fermentation with *Trichoderma viride*. In a related fermentation process with *P. ostreatus*, Villas-Bôas et al. (2003) observed an increase in the level of

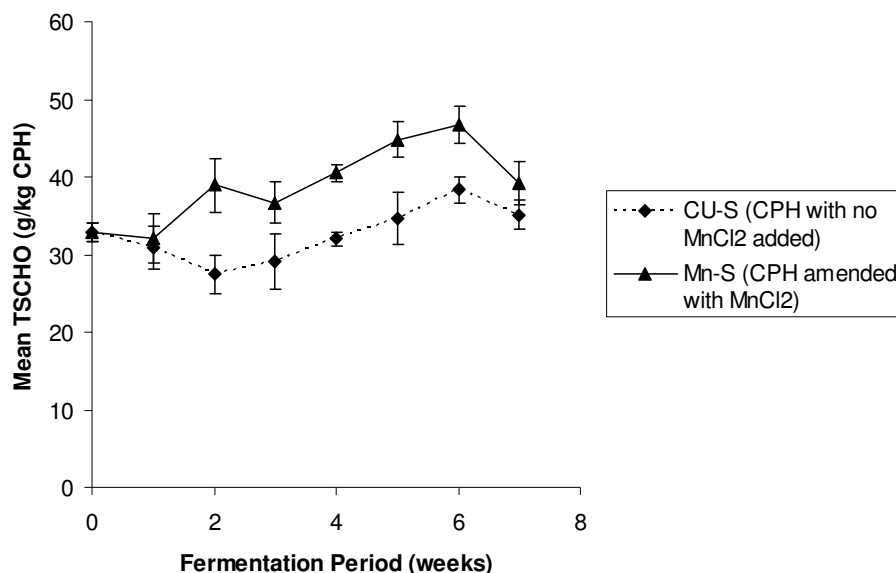


Figure 7. Changes in total sugars (TSCHO) during *Pleurotus* solid-state fermentation of CPH formulated substrates.

free sugars of apple pomace, which was attributed to degradation of polysaccharides such as pectin and hemicellulose. Increased soluble carbohydrate content of CPH following the fermentation will augment available energy of the substrate. The results also showed greater release of sugars from CPH when treated with Mn before fermentation ($P < 0.05$; Figure 7).

Optimising manganese amendment for *Pleurotus* fermentation of CPH

Since dietary concentrations of Mn ($MnCl_2$) at ≥ 4000 ppm have been reported to be toxic to poultry (Southern and Baker, 1983; NRC, 1994), the effect of lower Mn amendment levels on the composition of CPH was investigated. Figure 8 shows that within the optimum fermentation period of 5 weeks, Mn amendment levels of 250–10000 ppm all enhanced the bioconversion with 32–40% increase in protein and 14–40% increase in total soluble carbohydrates. Significant reductions in crude fibre (14–19%) and lignin (16–20%) were also observed at Mn concentrations ranging between 750–10000 ppm, but with no significant difference seen among the various concentrations. It can therefore be concluded that a Mn amendment level of 750 ppm will be optimum concentration to use; this value is well below the 4000 ppm limit for toxicity.

Effect of *Pleurotus* fermentation on polyphenolic fractions of CPH

Figure 9 shows that the tannin content of CPH was

reduced significantly ($P < 0.05$) by 93.28% after optimum *Pleurotus* solid-state fermentation. Leifa et al. (2006) reported a significant 79.19% reduction in tannin concentration of coffee husk after *Pleurotus* fermentation. High level of tannin in feedstuffs produces an anti-nutritive effect on nutrient digestibility and utilisation in animals and thus its reduction by *P. ostreatus* would partly enhance the nutritive value of CPH.

General implications of study

The present results demonstrate an improved composition of fibrous CPH as a result of its fermentation with *P. ostreatus*. The significant reduction in fibre and lignin contents coupled with increases in protein and soluble carbohydrates could be regarded as positive from the standpoint of improved feed quality of CPH. However on-farm growth performance study involving feeding *Pleurotus*-fermented CPH based diets to livestock, particularly poultry and pigs, needs to be conducted to further validate and support improved feed value of CPH as indicated by results of the present study. A feeding experiment with broilers would soon be conducted.

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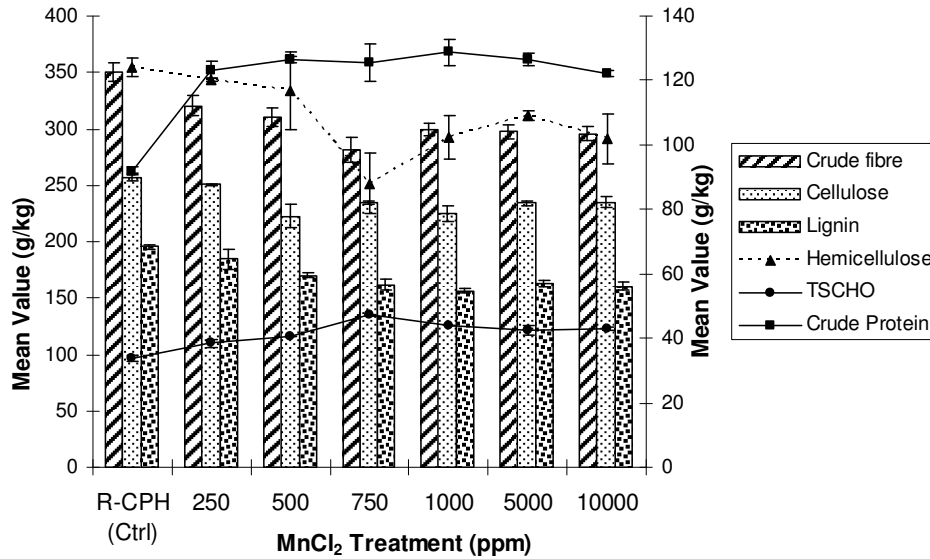


Figure 8. Effect of varying concentrations (≤ 10000 ppm) of manganese pre-treatment on CPH composition after 5-week *P. ostreatus* solid-state fermentation. R-CPH = Raw unfermented CPH (control).

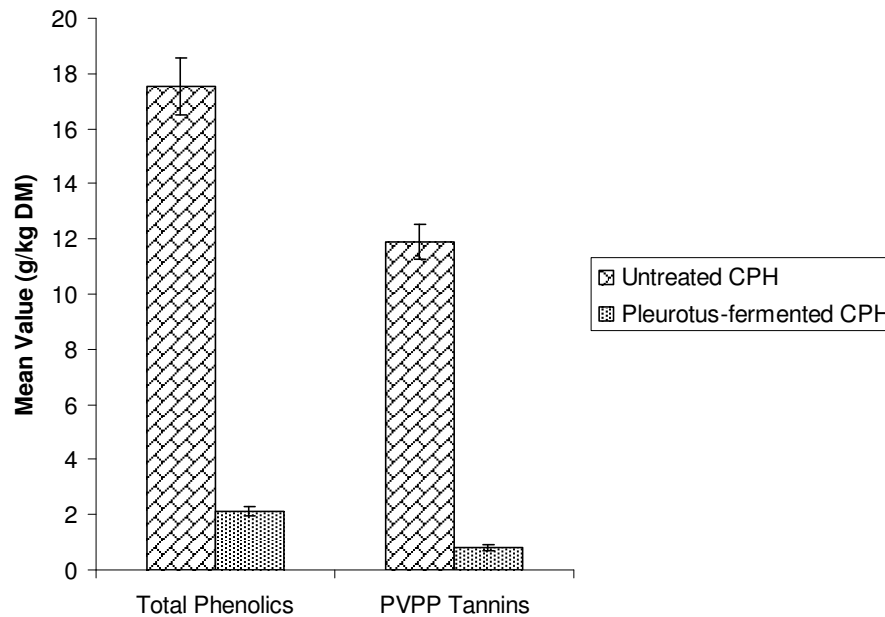


Figure 9. Total Phenolics and Tannin Content of CPH Before and After Optimum *Pleurotus* Fermentation

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