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# Effect of Lignin Peroxidases Obtained From White Rot Fungi in Delignification of Cereal Crop Residues for Ruminant Feeding : Changes in Chemical Composition And in Vitro Digestibility.

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***Abstract:*** *Crop residues are renewable sources of energy for ruminants. Pre-digestion of such materials with ligninases from white rot fungi may transform the lignocellulosic substrate into a feed with greater digestibility and higher quality for ruminants. This study has evaluated the effect of crude (T1) and purified(T2) lignin peroxidase (LiP) obtained from immobilized white rot fungi (LPS1) on chemical composition and in vitro digestibility of nine cereal crop residues commonly used for feeding ruminants. Untreated straw served as control(C). Each straw was hand chaffed into 2.3 to 3.0 cm bit length and treated with the enzyme by spraying at a ratio of 1:2.5 and left for 24 h before analysis. Significant P and F values at 99% CI respectively were obtained in case of ADF, NDF and ADL with both T1 and T2. Also in vitro digestibility of dry matter (IVDMD) increased significantly in both T1 and T2. Highest increase of 20 % in IVDMD was obtained upon treatment of BRM, FXM and PRM with purified lignin peroxidase (T2) while LM showed the lowest of 12.34 % . T2 thus showed higher digestibility than T1 for all the crops as shown by LS means. Correlation graph with digestibility on Y-axis and lignin degradation on X-axis showed a strong negative correlation for all the crop residues used with increase in digestibility giving a linear decrease in lignin content or vice versa. Also digestibility and lignin degradation differed for each type of straw evaluated. BRM and LM showed a very strong negative correlation (correlation coefficient r= -98.54 and -98.07) with FXM (r = -92.43) being the next in line followed by FMS (r=-87.67) and BA (r=-87.39). PRM, MS and PS followed with JR (r= -66.54) at the end. High yield of LiP obtained through immobilization on PUF cubes was effective in delignification and could be employed for enhancing the digestibility of crop residues.*

***Keywords:*** *white rot fungi, lignin peroxidase, cereal crop residues, lignin , digestibility*

## I. Introduction

If utilized judiciously, cereal crop residues could serve as effective feed for ruminants, on account of their vast energy content. The use of the polysaccharides, in these lignocellulosic complex is, however, limited on account of their high lignin content which is resistant to most of the bacterial enzymatic systems, as well as non-digestible by ruminants and rumen microflora.. Lignin removal is thus important in improving the digestibility and nutritive quality of these ruminant feed. Biological alternatives, to remove lignin from plant residues are highly promising because they are environmentally benign and widely acceptable.White-rot basidiomycetes (WRF ) and some actinomycetes are able to produce lignin-degrading enzymes, especially peroxidases which are capable of forming radicals inside the lignin polymer, leading to destabilization of bonds and finally in the breakdown of the macromolecule of lignin (Leonowicz *et al*., 1999). The ligninolytic enzymes attack lignin directly and thereby are the most promising long term alternatives to lignin removal by physical and chemical processes. White rot fungi selectively and efficiently degrade lignin by help of their ligninolytic enzymes comprised mainly by laccase, lignin peroxidase and manganese peroxidase, along with many other enzymes (Arora et al., 2002). None of the substrates i.e. lignin, cellulose or hemicelluloses is capable of being degraded by a single enzyme.

Lignin peroxidase (LiP, ligninase, (3,4dimethoxyphenyl) propane-1,3-diol:hydrogenperoxide oxidoreductases; EC 1.11.1.14) is the first oxidative enzyme discovered in *Phanerochaete chrysosporium* (Glenn *et al*., 1983 ). It is capable of catalyzing the depolymerization of the aromatic polymer lignin and a variety of non-phenolic lignin model compounds in the presence of $H_2O_2$ (Teunissen and Field, 1998; Haglund, 1999; Pérez *et al*., 2002; Hammel and Cullen, 2008).Lignin peroxidases are heme-containing glycoproteins and play a central role in the biodegradation of the cell wall constituent, lignin (Piontek et.al., 2001). LiPs catalyze the $H_2O_2$-dependent oxidative depolymerization of a variety of compounds ( tenHave and Teunissen, 2001) holding immense potential in biodelignification of crop residues. Earlier we reported enhancement in the nutritive profile and digestibility of finger millet straw *(Eleusine coracana*) upon treatment with crude extracts of lignolytic enzymes harvested from WRF (Sridhar *et al*., 2014). Subsequently, the beneficial effect of laccase enzyme produced by three wild isolates of white rot fungi in delignification and enhancing *in vitro* digestibility of *Eleusine coracana* was also successfully proved ( Kumar et.al, 2013). There are practically very few reports on changes in composition of crop residues after treatment with lignolytic enzymes in general and lignin peroxidase in particular. In this study we report the effect of lignin peroxidases isolated from wild WRF on selective ligninolysis and *in vitro* digestibility of nine cereal crop residues commonly used for feeding ruminants.

## II. Materials And Methods

### Collection of fungal isolates and Screening for Lignin peroxidase

Fruiting bodies of twenty six wild Basidiomycetes wood rotting fungi were collected in clean polythene bags from western Ghats of Coorg, Agumbe, Madikere and in and around Shimoga of Karnataka state and were grown by tissue culture (Revankar and Lele, 2006). These fruiting bodies were surface sterilized, inoculated into mycological agar slants followed by an incubation of 15-20 days at $30^0$C. Mycological agar (MA), Potato dextrose agar (PDA), Malt extract agar (MEA) & oat meal agar (OMA) containing 0.1% (W/V) of penicillin & streptomycin were used to isolate fungi based on the differences in growth patterns**.** Microscopic features showed characteristic features belonging to basidiomycetes. Thirty five commercial isolates were obtained from MTCC ,Chandigarh and NCIM, Pune. All the cultures were screened for lignin modifying enzymes (LME) by growing them on 0.02% Guaiacol supplemented LME basal medium. To further confirm the presence of lignin peroxidase, screening was also carried out using potato dextrose agar medium supplemented with 0.1% 3,3-dimethoxybenzidine. Each plate was inoculated with fungal agar plug of 7 mm (dia), from a fully grown fungal colony. The plates were incubated for 5-7 days at $30^0$C and formation of brown color was observed. Enzyme production from positive cultures in plate tests were also quantified by spectrophotometric assays .

### Immobilization and Purification of LiP

The stock culture of selected fungi were maintained on potato dextrose agar (PDA) media at 4 ºC and sub cultured every five days. Young cultures were grown on potato dextrose broth for 8 days at 39 ºC. The fungal biomass from these was used as the inoculums for immobilization on polyurethane foam (PUF) cubes cut manually into cubes (1x1x1cm). The selected strain was cultivated in submerged fermentation (Krishna Prasad et.al.,2005) under aseptic conditions at room temperature (28 $\pm 4^0$C) under continuous shaking on a orbital shaker (120 rpm). LiP activity was monitored regularly. The cell free extract of the fungus on day seven was taken as the crude LiP preparation (T1) .

For obtaining purified LiP (T2) **,** culture media from the culture flasks was harvested on day seven (maximum activity ) and precipitated using 70% $(NH_4)_2SO_4$. The solution was dialyzed overnight against 0.1 M sodium phosphate buffer (pH 6.8) and loaded onto a Sephadex G-50 column (44×3cm) equilibrated with the same buffer. The loaded proteins were eluted with the same buffer, at a flow rate of 1mL $^{-1}$ min. Collected fractions were assayed for both protein and LiP enzyme activity .Active fractions were pooled and concentrated on an Amicon PM 10 membrane (Millipore) and stored at - 20 °C and used for studying the changes in chemical composition and *in vitro* digestibility of the cereal crop residues .

**Treatment of cereal crop residues with LiP for changes in chemical composition and in vitro dry matter digestibility**

Straws of cereal crops commonly used to feed ruminants *viz.* Finger millet (FMS), Little millet (LM),Bajra (BA),Barnyard millet (BRM), Paddy(PS) , Maize (MS), Jowar (JR),Foxtail millet(FXM) and prosomillet (PRM) were procured locally from farmers, manually chaffed into 2 cm length, washed and dried at 100±5°C . LiP's obtained after immobilization to enhance production (T1) and after purification(T2) were used to treat the straws by spraying at an enzyme to straw ratio of 1:2.5 (Sridhar *et al*., 2014). Enzyme was sprayed and kept at room temperature for 24 h and used to study changes in the proximate composition and the digestibility . Untreated straws served sprayed with only production media served as control.

**Enzyme assays**

LiP activity in culture media and during purification was assayed by measuring the rate of $H_2O_2$ dependent oxidation of veratryl alcohol to veratraldehyde (Tien & Kirk, 1988). The assay contained 0.8mM veratryl alcohol in 0.1 M sodium tartarate buffer (pH 3.0). To this 1 ml of culture filtrate buffer was added in presence of 150 mM hydrogen peroxide. The linear absorbance was read at 310 nm for 1 min at $30^{0c}$. One unit of LiP was defined as 1 umol of veratraldehyde formed per minute and was expressed as U/ml (E = 9300 M-I cm").

The Dye B Azure method ( Arora & Gill, 2001) was used for estimating the enzyme during the straw treatment studies. The reaction mixture contained 1 ml of 125 mM sodium tartarate buffer (pH-3.0), 500 µlt of 0.160 mM Azure B dye, 500 µlt of the culture filtrate and 500 µlt of 2mM $H_2O_2$. The reaction was started by adding $H_2O_2$ and one unit of the enzyme activity was expressed as O.D decrease of 0.1 units/min/ml of the culture filtrate. MnP was estimated from the difference in absorbance maximum of substrate and products (Glen and Gold, 1985). Laccase activity was detected by the oxidation of ABTS at 4°C( Bourbonnais et al.,1998 ). Activities of carboxymethylcellulase (CM Case), Filter paper degrading activity, micro crystalline cellulose (MCC) and xylanase were estimated as per Agarwal *et al.* (2000) along with amylase (Safarik 1991), ß-glucosidase (Shewale and Sadana ,1978) and acetyl esterase (Martínez *et al.* 2007). Total proteolytic activity was measured by the procedure of Blackburn (1968).All enzyme assays were carried out in three replicates .

**Chemical Analysis**

After the samples were dried and milled ,chemical analyses were performed in three replicates. Dry matter was determined after drying at 100±5°C for 8 h. Nitrogen (N) content of the chaffed dried samples before and after fermentation was determined by the standard Kjeldhal method (AOAC 2000) and the crude protein (CP) was calculated (N × 6.25). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined using the method described by Van Soest *et al.* (1991). Protein of harvested enzyme media was estimated as per the method of Lowry *et al.* (1951) using BSA *In vitro*

dry matter digestibility (IVDMD) was determined using triplicate samples by the method of Tilley and Terry (1963).

**Statistical analysis**

The data for various parameters were tabulated and mean, standard deviation values were calculated according to Steel and Torrie (1980). The changes in distribution of fiber fractions with respect to different treatments were analyzed by SAS 9.3 software. The p and t values were sorted out by using Graph pad software.

## III. Results

**Collection of fungal isolates and Screening for Lignin peroxidase**

Potato dextrose agar (PDA), showed best growth in both commercial and wild isolates of White rot fungus (WRF). Qualitative screening for lignin peroxidase with Guaiacol and 3,3-dimethoxybenzidine showed development of maximum color in three of the screened isolates confirmingthe presence of lignin peroxidases (Fig 1).These wild isolates were designated as LPS1,LPS2 and LPS3 respectively.The accepted method for quantitative estimation of LiP is the spectrophotometric colorless veratryl alcohol assay method which was found to be superior compared to Azure B method.The latter is more economic & free from interference, and though it gives lower values compared to the veratryl alcohol assay method, it was adopted for the *in vitro* studies as phenolics from straw interfere in estimation of LiP .The highest activities of LiP recorded were 0.22±0.04 (22U/mL) in LPS1 ,0.19±0.04(19 U/mL) for LPS2. LPS3 had comparatively lower LiP activity of 0.13±0.03(13 U/mL) .

**Immobilization and Purification of Lignin peroxidase**

Immobilization of the selected two cultures on Poly Urethane foam showed enhanced activities of 0.690 (69 U/mL) and 0.661 (66 U/mL ) in LPS1 & LPS2 respectively on the 6-8$^{th}$ day of incubation (Fig 2). LPS1 showed highest activity of lignin peroxidase and was selected for further purification. The enzyme was purified 21 fold with a specific activity of 7.896 U/mg protein and yield of 69 % employing ammonium sulphate fractionation and gel filtration chromatography on Sephadex-G- 75. Enzyme activities of the crude LiP extract (T1) and after purification (T2) showed absence of all other fibrolytic enzymes in the purified extract (Table 1).

**Treatment ofcereal crop residues with Lignin peroxidase Changes in chemical composition**

The DM loss was minimal (1–2%) and protein did not differ significantly after treatment as compared to control in all the cereal crop residues (Fig. 3) evaluated in this study. The changes obtained in the composition of the fiber fractions of the nine crop residues upon treatment with crude (T1) and purified lignin peroxidase (T2) by spraying at an enzyme to straw ratio of 1:2.5 given in Table 2 showed significant P and F values at 99% CI respectively in case of ADF, NDF and ADL. The maximum reduction of 22.98% in ADF content was observed in PRM and minimum of 0.67 % in BA upon treatment with the crude enzyme. This was further reduced by 5.16 % to 28.14% in the former and by1.31 % to 1.98% in the latter upon treatment with purified lignin peroxidase. With regard to NDF content treatment of FXM with crude enzyme recorded the highest decrease of 7.38 % while the lowest decrease of 2.48 % was obtained in LM.PRM elicited the highest decrease of 15.39% in NDF content upon treatment with purified LiP while BA showed the lowest decrease of 3.96%. The ADL content showed a very marginal reduction of 0.39 % in JR and maximum of 1.2 % in BRM when treated with the crude LiP .Upon treatment with the

purified enzyme a further reduction of 2.86 % in ADL was recorded in JR. The interaction for ADF, NDF and ADL plotted in case of control, treated with crude LiP (T1) and purified LiP (T2) of the nine different cereal crop residues is clearly evident from Fig 3A, 3B and 3C .

**Changes in the *in vitro* dry matter digestibility**

The changes obtained with regard to the *in vitro* dry matter digestibility (IVDMD) of the nine crop residues upon treatment with crude (T1) and purified lignin peroxidase (T2) by spraying at an enzyme to straw ratio of 1:2.5 ( Table 2) showed significant P and F values at 99% CI. There was an increase in IVDMD % indicating enhanced digestibility of both treatments T1 and T2 in comparison with control. IVDMD showed significant variation upon treatment with LiP in comparison to control with F values 584.24 at 99% CI respectively($P<0.0001$). T2 showed higher digestibility than T1 for all the cereal crops, which is evident from the LS means. The maximum improvement of 20 % in IVDMD was obtained upon treatment of BRM, FXM and PRM with purified lignin peroxidase while LM showed the lowest enhancement of 12.34 % reflecting approximately a 3% increase in digestibility in these crop residues to that obtained upon treatment with the crude enzyme. The interaction for IVDMD plotted in case of the nine cereal crop residues treated with crude LiP (T1) and purified LiP (T2) against control (Fig 3D) clearly reflects the increased digestibility.

**Correlation between lignin content and IVDMD**

The relationship between digestibility and lignin degradation of the various straws (%) upon treatment with lignin peroxidase (LiP) was established by plotting a scatter graph using proc CORR of SAS (9.3). The ADL and IVDMD values obtained upon treatment of the nine crop residues with both crude (T1) and purified (T2) LiPs were considered for the study. Correlation was established in terms of lignin degradation and *in vitro* digestibility (Fig 4). Correlation graph clearly shows that with increase in digestibility there is a linear decrease in lignin content or vice versa. Correlation graph with digestibility (%) on Y-axis and lignin (%) on X-axis showed strong negative correlation for all the crop residues used. BRM and LM showed a very strong negative correlation (correlation coefficient r= -98.54 and -98.07) with FXM (r = -92.43) being the next in line followed by FMS (r=-87.67) and BA (r=-87.39). PRM, MS and PS followed with JR (r= -66.54) at the end.

## IV. Discussion

A characteristic feature of cereal crop residues is the refractory nature of their highly lignified cell walls. The breakdown of the bonds between lignin and cellulose, and particularly hemicellulose transforms these lignocellulosic substrates into feeds of high-quality for ruminants (El-Nasser et al., 1997). Microbial degradation of lignocellulosic materials brings a variety of changes in their bio-physicochemical properties. Mainly microbial enzymes such as xylanases, pectinases and endoglucanases have been applied to release cellulose fibers (Sørensen et al., 2004; Eun et al., 2006). On the basis of lignin degradation, ligninolytic fungi can be classified into three categories (a) simultaneous, (b) nonselective and (c) selective lignin degrading fungi. Being selectively lignin degraders, the white rot fungi are the most potential candidates, to improve the nutritional quality of these residues by degrading lignin and converting complex polysaccharides into simple sugars. Several white rot fungi have been evaluated for their potential to degrade lignocellulosics and their resultant effect on digestibility They are known to attack initially on the hemicellulose lignin matrix (Martınez et al., 2005) using xylanase, esterase and other ligninolytic enzymes; the esterase cleaves covalent bonds between polysaccharides and lignin (Dong et al., 2013).

The ligninolytic enzyme systems is comprised mainly of laccase, lignin peroxidase and manganese peroxidase, along with a few other enzymes (Arora et al., 2002; Arora & Sharma, 2010).

**Treatment of cereal crop residues with Lignin peroxidaseChanges in chemical composition**

The nutritive quality of cereal straws is correlated to their chemical composition which in turn governs their fiber degradation profile (Sharma and Arora, 2011). Fungal fermentation as in solid state conditions results in loss in DM as energy components utilized for growth. The longer the duration of the process, the higher the loss in DM leading to spent straw devoid of nutrients. However , direct application of enzyme extracts obtained from fungi for treating crop residues prevents the excessive dry matter loss encountered during fermentation. Kumar et al. (2015) evaluated the delignification of five common crop residues by laccase produced from immobilized *Schizophyllum commune* NI_07 as well as the one obtained in submerged culture and reported minimal DM losses as also was the case observed by us.

Fermentation is accompanied by enrichment or an increase in protein content of the substrate due to solubilization and degradation of fungal protein or hydrolysis of starch to glucose and its subsequent use by the fungi as a carbon source to synthesize biomass rich in protein (Belewu and Belewu, 2005). No significant variations were obtained with regard to the protein content of the cereal crop residues after treatment as the crude extract(T1) and purified enzyme(T2) only added protein to the cereal crops but did not cause changes in chemical composition unlike those observed in the case of fermentation with fungal biomass. In our study ADF, NDF and ADL showed significant variation upon treatment with LiP in comparison to control. Decrease in ADF, NDF and ADL% indicates increase in digestible energy levels along with lignin degradation of both treatments T1 and T2 in comparison with control. T2 showed higher degree of degradation than T1 for all the cereal crops, which is shown by LS means.

A preliminary investigation was conducted to assess lignocellulolytic efficiency of crude extracts from three white-rot fungi, *Pleurotus florida* , *Pleurotus sajor-caju* and *Pleurotus eryngii* . The activities of CMC-ase, xylanase, β-glucosidase, β -xylosidase, laccase and Mn peroxidase in extracts were evaluated and employed for their *in vitro* degradability assessment to corn cob substrate. The losses in cell wall components and dry matter during 5 and10 days incubations showed maximum 8.2, 4.4 and 2.8% loss in hemicellulose, cellulose and lignin with mono extract of *Pleurotus florida* within 10 days. The influence of mono extract of individual strains and their mixed extracts on degradation of cell wall constituents differed remarkably.The mixed extract treatment recorded maximum loss of hemicellulose (13.6% ) , 9.2% loss in cellulose and 5.2% loss of lignin. The highest dry matter loss (8.2%) was also recorded with the mixed extract combination (Naraian et al, 2010).

Sridhar et al., (2014) evaluated the effect of different doses of three exogenous lignolytic enzymes - laccase (Lac), lignin peroxidase (LiP) and manganese peroxidase (MnP) obtained from immobilized *Pleurotus flabellatus, Poria placenta* and *Coriolus versicolor (Polystictus versicolor)* on the nutritional profile and in vitro digestibility of ragi straw. Ragi straw of 2.3 to 3.0 cm length was supplemented as well as treated for 24 h by spraying with this enzyme rich media harvested from each individual fungi at varying ratios of 1:5; 2:5 and 3:5. No significant changes ($p>0.05$) were obtained either in cell wall components (NDF, ADF ADL) or the *in vitro* digestibility with any of the fungi upon supplementation with the lignolytic enzymes. However upon treatment with lignolytic enzymes at a ratio of 2:5, there was a significant change in the chemical composition as well as the *in vitro* dry matter digestibility of ragi straw. Kumar et al.,( 2013) observed a decrease in NDF content of ragi

straw (2cm to 3 cm length) treated with laccases of three white rot fungi NI-07, NI-04 and NI-09. The improvement in the quality of straw fermented with white rot fungi was attributed to the degradation of lignin besides the production of protein (Kamra and Zadrazil,1988). An increase in lignin content affects directly the degradation of neutral detergent fibre (Caballero et al., 2001). Though lignin content increases at a higher rate than the NDF content, degradation of dry matter was correlated well with NDF digestibility than with NDF content (Dechamps,1999). The lignocellulosic substrates are transformed into high quality feed for ruminants on account of the breakdown of bonds between lignin and cellulose (El-Nasser et al., 1997). The lignin peroxidases from white rot are found to be more effective in feed digestibility than the endoglucanases.

**Changes in in vitro dry matter digestibility**

To obtain a quick and precise prediction of in-vivo digestibility in ruminants, digestibility measured by in-vitro methods is employed as it gives a fairly close idea about the quality of feed (Goering & Van Soest, 1970) . An increase in dry matter digestibility shows increased quality of feed and less feed intake. The two stage in-vitro procedure developed by Tilley & Terry (1963) is the most reliable laboratory based method for predicting the digestibility of a wide range of forages. It can predict in-vivo digestibility with a lower error than any chemical method and has been widely accepted throughout the world for measuring the digestibility of feeds (Minson, 1990; Shrivastava et al., 2012). Several workers have demonstrated successful bioconversion of lignocellulosic residues into nutritive animal feed using white rot fungi under solid state degradation . Cohen et al. (2002) observed that during selective lignin degradation, cellulose was exposed and could be utilized by ruminants. This strengthens the viewpoint that delignification plays an important role in improvement of the digestibility and feed value of straw. Increase in IVDMD % obtained upon treatment of the crop residues with LiPs indicates enhanced digestibility of both treatments T1 and T2 in comparison with control. Digestibility and lignin degradation differed for each type of straw used.

**Correlation between lignin content and IVDMD**

Cell wall constituents of straw play an important role in determining its quality as animal feed. Lignin being a phenolic biopolymer is difficult to be digested by ruminants. Higher lignin and tannin content results in lower digestibility of lignocellulosics and plant residues (Arigbede et al., 2012). As evident from earlier observations, a strong negative correlation existed between lignin content and in-vitro digestibility of undecayed paddy straw samples, while a strong positive correlation was observed between lignin loss and in-vitro digestibility of degraded straw (Arora & Sharma, 2009b; Sharma & Arora, 2011). Hence it can be safely said that lignin peroxidase obtained in the present study was effective in lignin degradation of cereal crop residues which was reflected in the changes in the chemical composition and enhancement of *in vitro* dry matter digestibility.

## V. Conclusion

The results of the present study successfully demonstrated the effects of lignin peroxidase of white rot fungi in lignolysis and enhancing the digestibility of crop residues . Purified lignin peroxidase was found to be more effective in increasing the digestibility along with decrease in lignin content of all nine straws. Irrespective of different straws and their lignin content, both crude and purified enzyme significantly influenced lignin degradation Application of lignolytic enzymes as feed supplements promises to be a simple but effective technology for achieving increased digestibility of crop residues and enhancing animal

productivity in the near future. However, *in vivo* feeding trials are deemed essential, to corroborate the results of the present work.

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**Table 1:** Enzyme activities of the crude LiP extract (T1) and after purification (T2)

| **Enzyme** | **Enzyme Treatment** | |
|---|---|---|
| | **T1** | **T2** |
| eroxidase[1] | 0.68±0.12 | 2.95±0.35 |
| nganese Peroxidase[2] | 0.19±0.05 | ND |
| | ND | ND |
| Xylanase [4] | 64 ±17.69 | ND |
| Amylase[4] | 58 ± 9.8 | ND |
| CM cellulase[4] | 59.33 ± 9.01 | ND |
| Micro crystalline cellulase[4] | 4.7 ± 0.29 | ND |
| Filter paper degrading activity[4] | 3.30 ± 0.52 | ND |
| β - glucosidase[5] | 1.02 ± 0.40 | ND |
| Acetyl esterase[5] | 1.02 ± 0.30 | ND |
| Protease[6] | 486 ± 22 | ND |

[1]One unit (U) of Lignin Peroxidase activity defined as activity of enzyme that catalyzes the conversion of 1µmole of veratryl alcohol per minute, [2]One unit of manganese peroxidase expressed as activity of enzyme that catalyzes the conversion of 1µmole of DMP per minute,[3]The activity of enzyme that catalyzes the conversion of 1µmole of 2,2'-azino-bis(3-ethyl benzothiazoline)-6-sulphonic acid (ABTS) per minute.[4] One unit of enzyme activity expressed as mgs of reducing sugar liberated ml/hr.[5]One unit of enzyme activity expressed as µgm of P-nitrophenol liberated ml/hr. Values are averages of estimations carried out in three replicate

**Table 2 :** Changes obtained in the composition of fiber fractions and digestibility of cereal crop residues upon treatment with crude LiP (T1) and purified LiP (T2)

| **Straw** | **Groups** | **ADF[1]** | **NDF[2]** | **ADL[3]** | **IVDMD[4]** |
|---|---|---|---|---|---|
| BA | C | 46.21±0.38 | 80.37±0.56 | 6.77±0.1 | 44.04±0.35 |
| | T1 | 45.54±0.38 | 77.12±0.56 | 6.17±0.1 | 57.65±0.35 |
| | T2 | 44.23±0.38 | 76.41±0.56 | 4.66±0.1 | 63.85±0.35 |
| BRM | C | 48.63±0.38 | 80.86±0.56 | 6.87±0.1 | 43.44±0.35 |
| | T1 | 45.23±0.38 | 74.59±0.56 | 5.67±0.1 | 60.5±0.35 |
| | T2 | 44.54±0.38 | 70.03±0.56 | 5.39±0.1 | 63.9±0.35 |
| FMS | C | 39.4±0.38 | 77.06±0.56 | 6.76±0.1 | 40.67±0.35 |
| | T1 | 34.9±0.38 | 73.72±0.56 | 5.63±0.1 | 45.61±0.35 |

| | | | | | |
|---|---|---|---|---|---|
| | T2 | 34.02±0.38 | 63.39±0.56 | 5.13±0.1 | 57.28±0.35 |
| FXM | C | 48.46±0.38 | 81.13±0.56 | 7.63±0.1 | 43.27±0.35 |
| | T1 | 45.07±0.38 | 73.75±0.56 | 6.79±0.1 | 60.91±0.35 |
| | T2 | 44.44±0.38 | 69.5±0.56 | 6.19±0.1 | 63.31±0.35 |
| JR | C | 46.86±0.38 | 80.89±0.56 | 7.07±0.1 | 43.28±0.35 |
| | T1 | 42.37±0.38 | 74.22±0.56 | 6.68±0.1 | 55.43±0.35 |
| | T2 | 41.49±0.38 | 71.47±0.56 | 4.21±0.1 | 56.77±0.35 |
| LM | C | 48.73±0.38 | 80.65±0.56 | 6.63±0.1 | 41.64±0.35 |
| | T1 | 31.19±0.38 | 78.17±0.56 | 5.46±0.1 | 52.55±0.35 |
| | T2 | 30.19±0.38 | 74.23±0.56 | 5.12±0.1 | 53.98±0.35 |
| MS | C | 49.52±0.38 | 79.69±0.56 | 6.24±0.1 | 42.59±0.35 |
| | T1 | 47.36±0.38 | 75.04±0.56 | 5.8±0.1 | 58.25±0.35 |
| | T2 | 39.67±0.38 | 72.88±0.56 | 5.4±0.1 | 61.96±0.35 |
| PRM | C | 66.35±0.38 | 81.79±0.56 | 6.89±0.1 | 39.96±0.35 |
| | T1 | 43.37±0.38 | 76.17±0.56 | 6.28±0.1 | 57.85±0.35 |
| | T2 | 38.21±0.38 | 66.4±0.56 | 5.49±0.1 | 60.14±0.35 |
| PS | C | 49.73±0.38 | 81.19±0.56 | 8.16±0.1 | 41.79±0.35 |
| | T1 | 47.41±0.38 | 76.68±0.56 | 7.23±0.1 | 55.85±0.35 |
| | T2 | 46.55±0.38 | 66.35±0.56 | 5.58±0.1 | 58.03±0.35 |
| **F Value** | | **345.06** | **79.86** | **84.04** | **584.24** |
| **P Value** | | **<0001**** | **<0001**** | **<0001**** | **<0001**** |

LS means ± standard error values, T1= treatment with crude LiP ,T2= treatment with pure LiP, Bajra-BA; Barnyard millet -BRM; Finger millet -FMS; Foxtail millet-FXM; jowar -JR; little millet -LM; Paddy-PS; Maize -MS; and Prosomillet -PRM. [1] Acid detergent fibre, [2]Neutral Detergent Fibre, [3] Acid detergent lignin, [4]*In vitro* dry matter digestibility,

**Legends for Figures:**

Fig 1: Plate screening of fungal isolates for presence of lignin peroxidase .

Fig 2: Changes obtained in the Lignin peroxidase activities of LPS1 and LPS2 upon immobilization for fourteen days.

Fig 3: Changes in the dry matter and protein (%) of the control and cereal crop residues upon treatment with crude LiP (T1) and purified LiP (T2).

Fig 4:Interaction plot for ADF, NDF , ADL and IVDMD between control, treated with crude LiP (T1) and purified LiP (T2) for different cereal crop residues.

Fig 5 : Correlation between in vitro digestibility (IVDMD) and lignin content (ADL) of the different cereal crop residues (%) upon treatment with lignin peroxidase (LiP)

**Fig 1:**

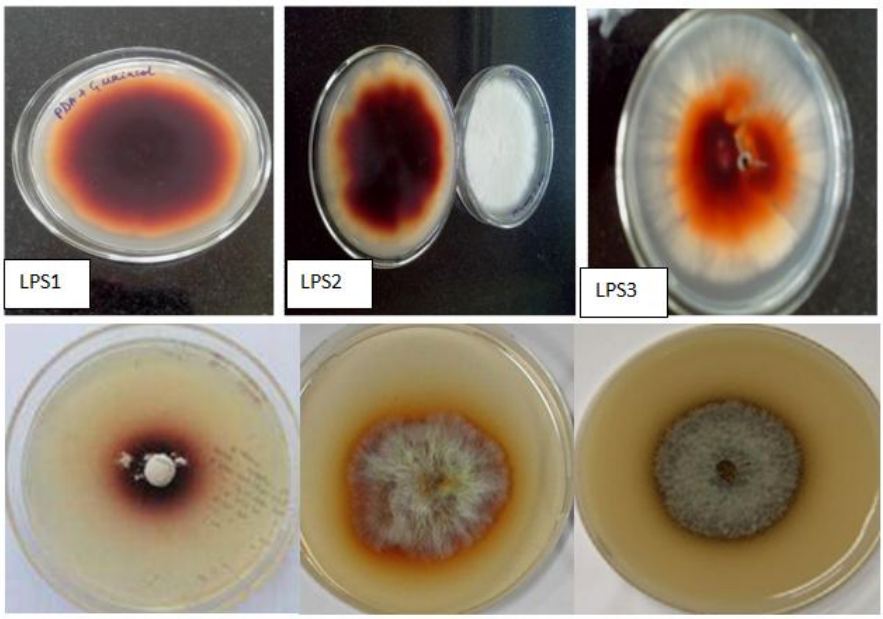



**Fig 2:** Immobilization of LPS1and LPS2 on PUF cubes

**Fig 2:**

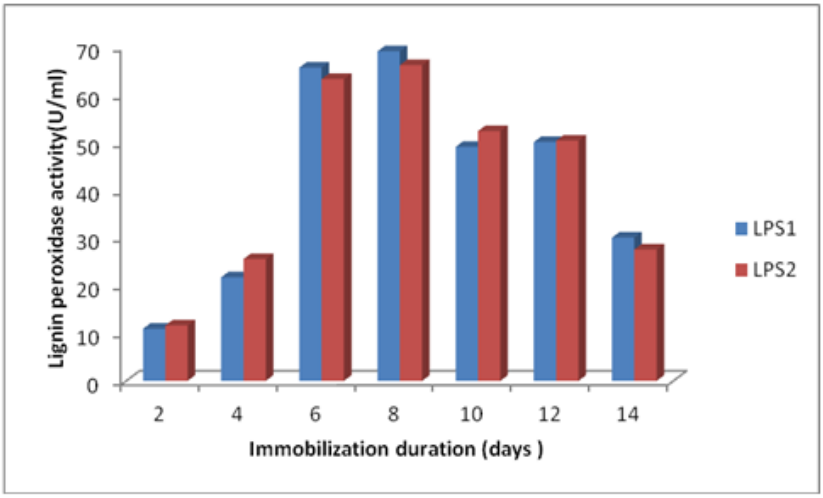



**Fig 3:**

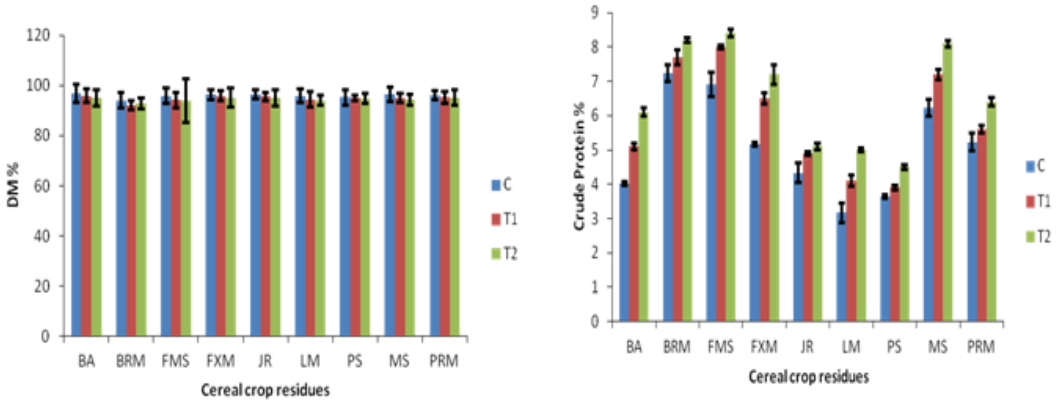
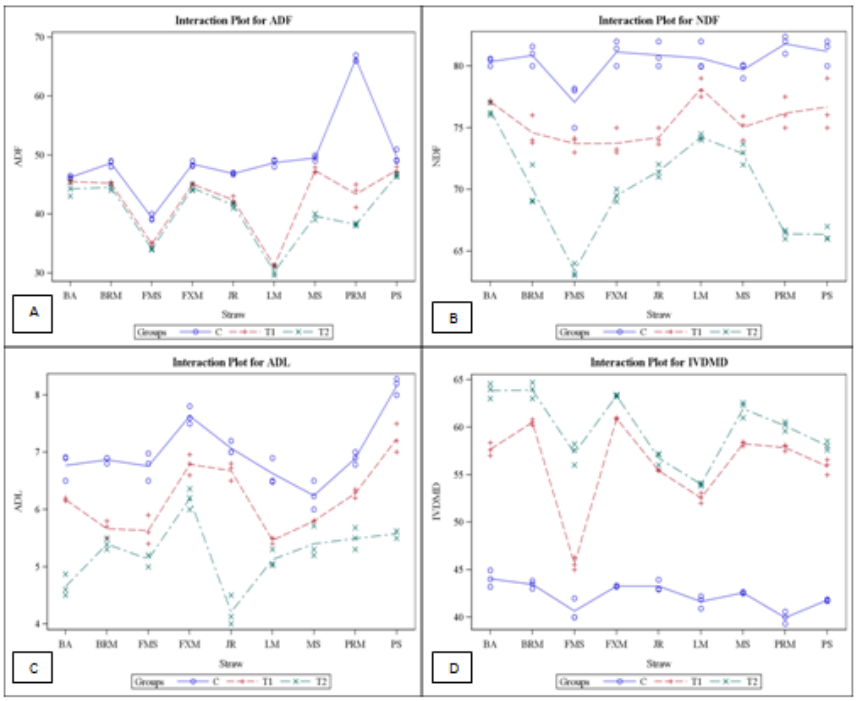
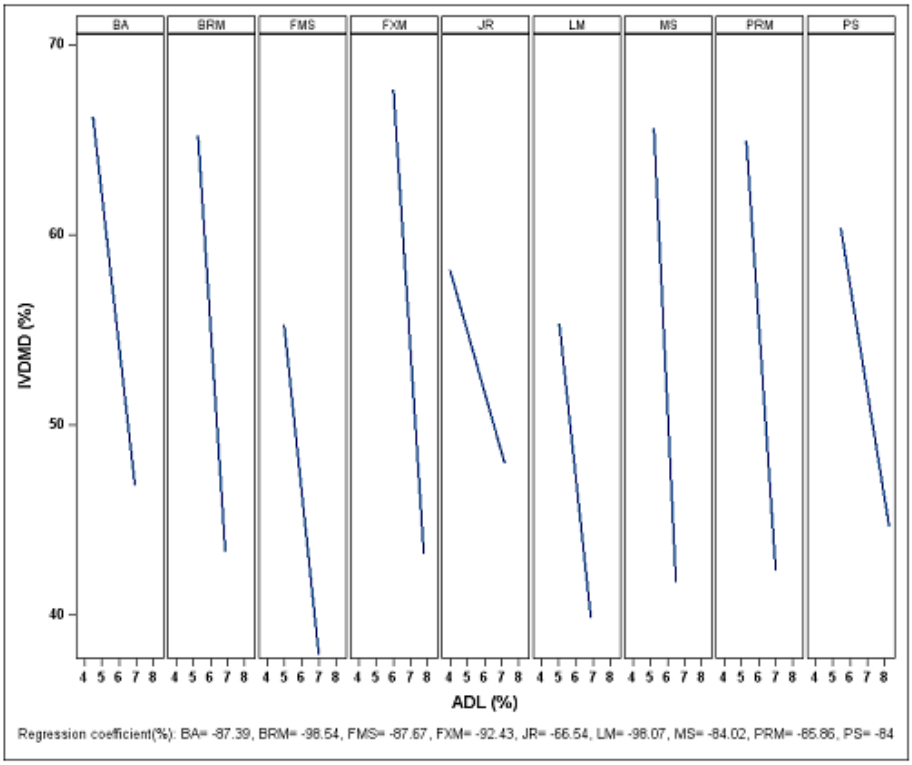



**Fig 4:**




Bajra-BA ; Barnyard millet -BRM; Finger millet -FMS; Foxtail millet-FXM; jowar -JR; little millet -LM; Paddy-PS; Maize -MS; and Prosomillet -PRM.

**Fig5 :**




Bajra-BA ; Barnyard millet -BRM; Finger millet -FMS; Foxtail millet-FXM; jowar -JR; little millet -LM; Paddy-PS; Maize -MS; and Prosomillet -PRM.