PJN
ISSN 1680-5194

Pakistan Journal of Nutrition

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2018.71.75

Research Article
Biodegradation of Lignin by White Rot Fungi (*Pleurotus ostreatus*) to Decrease the Fibre Components in the Palm Midrib

[1,2]Yurma Metri, [1]Lili Warly and [1]Suyitman

[1]Faculty of Animal Husbandry, Andalas University, Campus UNAND Limau Manis, 25163 Padang, Indonesia
[2]Department of Agriculture, Haji Agus Salim, Jalan Prof. Bachder Djohan Jangkak Mandiangin Bukittinggi, Sumatra Barat, Indonesia

Abstract
Background and Objective: Utilization of plantation waste, such as palm stems, is still limited due to its high lignin content and low digestibility. Therefore, it is necessary to apply technology to improve feed quality in terms of both nutritional value and waste digestibility. Such technology will ultimately improve the quality of animal feed. White rot fungi, such as the basidiomycete *Pleurotus ostreatus* (*P. ostreatus*), can degrade lignin by using lignases, which include lignin peroxidase, manganese peroxidase and laccase. **Materials and Methods:** The study was conducted in the Nutrition Laboratory of Andalas University. This study used completely randomized design with factorial pattern. Factor A is *P. ostreatus* dose (3, 5 and 7%) and factor B is the fermentation time (1, 2 and 3 months). **Results:** The results showed that fermentation of palm stem with *P. ostreatus* for 3 months could decrease NDF content up to 11.03%, ADF content up to 9.65%, hemicellulose content up to 1.39% and lignin content up to 7.24%. **Conclusion:** The dose of *P. ostreatus* and the fermentation time did not interact significantly. However, the fermentation time of 3 months significantly ($p<0.05$) decreased the contents of NDF, ADF, hemicellulose and lignin in palm midribs and can be used to improve the quality of animal feed ingredients, especially feed used for ruminants.

Key words: White rot fungus, lignin, palm midrib, feed quality, nutrition, biodegradation, fermentation

Received: September 04, 2017 Accepted: January 03, 2018 Published: January 15, 2018

Citation: Yurma Metri, Lili Warly and Suyitman, 2018. Biodegradation of lignin by white rot fungi (*Pleurotus ostreatus*) to decrease the fibre components in the palm midrib. Pak. J. Nutr., 17: 71-75.

Corresponding Author: Yurma Metri, Faculty of Animal Husbandry, Andalas University, Campus UNAND Limau Manis, 25163 Padang, Indonesia
Tel/Fax: (0752)71464

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The utilization of plantation waste, such as palm midribs, is constrained by low levels of digestibility due to the levels of neutral detergent fibre (NDF) and the high lignin content. The lignin levels of the palm fronds are 22.936%[1]. Lignin and crude fibre (cellulose and hemicelluloses) will form bonds to produce lignocelluloses. A high concentration of bound lignocelluloses in animal feed ingredients results in the production of cellulase and hemicellulases by rumen microbes that are unable to hydrolyse cellulose and hemicelluloses to produce volatile fatty acids.

Lignin is a complex aromatic polymer formed by the polymerization of three-dimensional cinnamyl alcohol (a propane phenyl derivative). Thus, lignin is a polyphenolic macromolecule. The lignin polymer can be converted to a monomer without any change in its basic form. Lignin that is complexed with cellulose is resistant to hydrolysis due to its bonds and the bound aryl alkyl ether[2].

Delignification can be performed using chemical or biological treatments. Chemical treatments, which use chlorine, sodium hydroxide, hydrogen peroxide or even ozone[3], can cause economic losses due to the impact of pollution on the environment. Biological treatment using microorganisms that produce enzymes has become a widely used alternative to chemical treatment[4].

High levels of lignin in animal feed can decrease the palatability, nutritional value and digestibility of the feed[5], so that the feed cannot be fully utilized by ruminants[6]. Thus, applying technology to improve the feed quality in terms of both its nutritional value and the digestibility of the waste will ultimately improve the quality of the meat. *Pleurotus ostreatus* is a fungus capable of reducing lignin content in wood weathering processes. Lignin degradation involves the activity of enzymes produced by white rot fungi. These enzymes, called lignases, include lignin peroxidases, manganese peroxidases and laccase[7].

Based on the type of weathering they cause, fungi involved in the biodegradation of the lignocellulose biomass can be divided into three main groups: White rot, brown rot and soft rot fungi. White rot and brown rot fungi are members of Basidiomycetes, while the soft rot fungi are members of Ascomycetes and their activities are often associated with high- and low-humidity timber, respectively[8]. Brown rot fungi degrade polysaccharides in the lignocellulose biomass and only slightly degrade the lignin. White rot fungi are the most efficient microorganisms at degrading lignin to CO_2 and H_2O[9]. Ligninolytic activity is associated with the production of extracellular enzymes by the white rot fungi that degrade lignin.

The white rot fungus *Pleurotus ostreatus* a basidiomycete, has economic value because it can be consumed and has a high nutritional content. The aim of this study was to assess the fermentation conditions necessary to enable the use of palm midrib as an ingredient for ruminant feed. Specifically, the interactions between the dose and duration of fermentation with inoculants were evaluated regarding their effects on the fibre components of palm midrib.

MATERIALS AND METHODS

The raw materials was used in this study include palm midrib and the white rot fungus *Pleurotus ostreatus* as an inoculant. The palm midrib was already chopped and dried and the fungus was cultured on PDA medium and then inoculated on immobilization medium in a 1:1:1 mixture of media sawdust, fine bran and corn flour.

Implementation of research: *Pleurotus ostreatus* that had been cultivated on the immobilization medium was inoculated using an inoculant dose that was further fermented in 1 kg of palm fronds in a cylindrical bag, hence, forth called a log-shaped bag. The duration of fermentation depended on the treatment (1, 2 or 3 months).

Parameters evaluated: The parameters evaluated in this study were components of the fibre fraction of palm fronds, neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose, cellulose and lignin. Each component was measured both before and after fermentation and the results were analysed using the method of Goering and Van Soest[10].

Statistical analysis: This study used a completely randomized factorial design with three replications per combination. The treatments in this study were as follows: *P. ostreatus* inoculum (3, 5 and 7%) and long fermentation (1, 2 and 3 months). Further tests were conducted using Duncan's new multiple range test (DNMRT) at the 5% level of significance.

RESULTS AND DISCUSSION

NDF content: Statistical analysis showed that there was no interaction between the *P. ostreatus* dose and the fermentation time. However, a long duration of fermentation had a significant ($p<0.05$) effect on the NDF content of the palm fronds. Table 1 shows that before fermentation, the NDF content of the palm midrib was 77.349% and this value continuously decreased at months 1-3 during fermentation,

Table 1: Fibre fraction contents of palm midrib before bio-fermentation with *P. ostreatus*

Nutrient	Content (%)*
NDF	77.349
ADF	66.193
Hemicellulose	11.156
Cellulose	37.196
Lignin	22.936

*Laboratory analysis results Ruminant Basic Nutrients, Faculty of Animal Husbandry, Andalas University in Padang, based on 100% dry matter

Table 2: NDF contents of palm midrib after bio-fermentation with *P. ostreatus*

Inoculant dose (%)	Fermentation time (months) 1	2	3	Average
0.3	75.782	71.342	70.363	72.496
0.5	75.719	70.749	64.618	70.362
0.7	73.380	70.138	66.317	69.945
Average	74.960[b]	70.743[a]	67.099[a]	
SE = 1.287				

The numbers in the same column followed by the same small letters are not significantly different at the 5% based on DNMRT DF analysis results after treatment

Table 3: ADF contents of palm midrib after bio-fermentation with *P. ostreatus*

Inoculant dose (%)	Fermentation time (months) 1	2	3	Average
0.3	59.621	57.550	58.933	58.701
0.5	60.414	59.162	56.412	58.663
0.7	60.702	58.467	56.545	58.571
Average	60.246[b]	58.393[a]	57.296[a]	
SE = 0.785				

The numbers in the same column followed by the same small letters are not significantly different at the 5% level based on DNMRT DF analysis results after treatment

Table 4: Hemicellulose contents of palm midrib after bio-fermentation with *P. ostreatus*

Inoculant dose (%)	Fermentation time (months) 1	2	3	Average
0.3	16.161	13.792	11.430	13.795
0.5	15.305	11.588	8.207	11.700
0.7	12.677	11.671	9.772	11.373
Average	14.715[b]	12.350[a]	9.803[a]	
SE = 1.063				

The numbers in the same column followed by the same small letters are not significantly different at the 5% level based on DNMRT DF analysis results after treatment

with values of 74.960, 70.43 and 67.099%, respectively (Table 2). While the dose of *P. ostreatus* used was not statistically significant, the higher dose resulted in a reduction in the content of NDF.

The decline in the NDF content confirms that the lignocellulases produced by *P. ostreatus* can hydrolyse the lignin and hemicellulose bonds so effectively that they were decomposed. The decrease in the NDF content results in a decrease in the content of substrate that then affects the composition of the fibres.

In this study, the NDF content of the palm midrib decreased because the white rot fungus disrupted the lignocellulose bonds during fermentation. The disruption of those bonds was also accompanied by the development of microbial activity[11].

ADF content: Statistical analysis showed no significant interaction between the dose of *P. ostreatus* and the fermentation time. However, long-term fermentation significantly decreased ($p<0.05$) the ADF content of palm midrib. Before fermentation, the content of ADF in the palm midribs was 66.193% and over the 3 months period, that value decreased to 57.296% (Table 3). The amount of fungus applied was non-significant, but on average, the dose of 0.7% resulted in a lower ADF content due to *P. ostreatus* activity.

The decrease in the ADF content in the palm midrib is due to the *P. ostreatus* enzyme that can disrupt the cellulose bonds. Thus, optimization of the fermentation process, resulting in the optimal production of cellulase, caused the disruption of previously strong bonds.

Studies that use different microbes with the same substrate can reduce the ADF content of the untreated substrate by 50.32%. In this study, fermentation for 30 days decreased the ADF content by 42.69%. The success of fermentation is strongly influenced by both the dose of inoculum and the fermentation time[11].

Hemicellulose content: Statistical analysis showed no significant interactions between the dose of *P. ostreatus* and the fermentation time in terms of the hemicellulose content. However, the duration of fermentation significantly affected ($p<0.05$) the hemicellulose content of the palm midribs. The hemicellulose content of the palm midribs before fermentation was 11.156% and it decreased to 9.803% after 3 months of fermentation with *P. ostreatus* (Table 4). Although the dose was not statistically significant, on average, it appears that the higher doses of *P. ostreatus* resulted in lower levels of hemicellulose.

The decrease in the hemicellulose content was correlated with the decrease in the lignin content over the course of the fermentation. Since the isolates of *P. ostreatus* degrade cellulose, the hemicelluloses are also degraded. The degradation of the lignin results in access to the cellulose and hemicelluloses, enabling alteration of their bonds. Reshuffling the components of cellulose will produce simple sugars. Degradation of cellulose limits the production of most enzymes that degrade hemicellulose. This process is performed by the white rot fungus.

Table 5: Cellulose contents of palm midrib after bio-fermentation with *P. ostreatus*

Inoculant dose (%)	Fermentation time (months) 1	2	3	Average
0.3	30.637	31.301	32.085	31.341
0.5	32.058	30.960	31.656	31.558
0.7	32.534	31.808	30.627	31.656
Average	31.743	31.356	31.456	
SE = 1.147				

The values in this table show no significant differences at the 5% level based on the F-test

Table 6: Lignin contents of palm midrib after bio-fermentation with *P. ostreatus*

Inoculant dose (%)	Fermentation time (months) 1	2	3	Average
0.3	22.705	20.576	20.147	21.143
0.5	19.837	18.585	16.524	18.315
0.7	19.936	16.987	15.703	17.542
Average	20.826[b]	18.716[a]	17.458[a]	
SE = 0.693				

The numbers in the same column followed by the same small letters are not significantly different at the 5% level based on DNMRT DF analysis results after treatment

A further explanation is that during the degradation of hemicellulose, the hemicelluloses were attacked first by endoenzymes (mannanase and xylanase), which resulted in the intensive hydrolysis of short bonds to simple sugars by various enzymes (mannosidase, xylosidase and glucosidase). As with the cellulases, the production of simple sugars limits the production of most of the enzymes of the white rot fungi that degrade hemicellulose. Cellulose is thought to be the source of the carbon that accelerates the formation of the fungal enzymes that degrade hemicellulose[12].

Cellulose content: Statistical analysis showed that the cellulose content of the palm midribs was not significantly affected by the *P. ostreatus* dose, the fermentation time or the interaction of those factors, although there were fluctuations in the average cellulose content. Before fermentation, the palm midribs contained 37.196% cellulose, this decreased to 31.743, 31.356 and 31.456% after 1, 2 and 3 months, respectively (Table 5). Although, the dose of *P. ostreatus* was not statistically significant, on average, it appears that the higher *P. ostreatus* dose resulted in a higher content of cellulose.

The cellulose content has a tendency to decrease with a longer incubation period because the fungus continues to degrade cellulose to generate substrates for its growth. *Pleurotus ostreatus* has been shown to degrade cellulose to compounds that it can more effectively use as substrates for growth[13].

The cellulose degradation peaked when *P. ostreatus* formed fruiting bodies, because at that time, different environmental conditions induce changes in fungal metabolism. Decreased levels of cellulase due to the increasing thickening of the mycelia can restructure the cellulose[14].

Long incubation times increase the concentration of the mycelium in the substrate. The optimal concentration of the mycelium produces cellulase more effectively, decreasing the content of cellulose. When the mycelia have grown thick and evenly cover the entire surface of the substrate, the concentration of the enzyme is high. These results in the degradation of the cell wall components, especially those of the fibres that then have their cellulose degraded[15].

Lignin content: Statistical analysis showed no significant interaction effect of the dose of *P. ostreatus* and the fermentation time on the lignin content. However, the fermentation time significantly ($p<0.05$) decreased the lignin content of the palm midribs. The lignin content of the palm midribs was 22.936% before fermentation but decreased to 17.458% after 3 months (Table 6). Although, the doses of *P. ostreatus* did not differ significantly, on average, it appears that the higher doses of *P. ostreatus* resulted in lower amounts of lignin present.

The levels of lignin in the bag log with *P. ostreatus* decreased because the fungus used lignin for growth. After 2 months of incubation with *P. ostreatus*, the fermentation has begun and the first harvest has occurred. This observation agrees with a previous report[16] which indicated that *P. ostreatus* belongs to a group of microorganisms that can thoroughly break down lignin to carbon dioxide and water. This group of fungi produces a family of enzymes that are directly involved in the restructuring of lignin, including the phenoloxidase laccase, lignin peroxidase (LiP) and manganese peroxidase (MnP). *Pleurotus ostreatus* includes spoilage white rot fungi that can degrade lignin and improve the digestibility of dry matter and organic ingredients such as rice straw[17].

Reduced lignin levels lead to the increased availability of nutrients such as dry matter and organic matter, allowing the palm midribs to be used as feed for ruminants. However, the bag log composed of palm midrib has a low nutrient content. Thus, more research is needed to produce technological breakthroughs that will increase its nutritional value.

CONCLUSION

The results showed that the dosage of *P. ostreatus* and the fermentation time did not interact significantly. However, the fermentation time of 3 months significantly ($p<0.05$) decreased the contents of NDF, ADF, hemicellulose and lignin palm midribs. It can therefore be used to improve the quality of animal feed ingredients, especially feed used for ruminants.

ACKNOWLEDGMENT

This study was funded by the Directorate General of Higher Education of Indonesia (DIKTI), Ministry of National Education, Ref. No. 48 / KONTRAK-PENELITIAN / 010.KM / 2016 (Dipa Kopertis Wil X).

REFERENCES

1. Metri, Y., 2016. The results of analysis in the laboratory of basic nutrition. Andalas University, Indonesia.
2. Fachry, A.R., P. Astuti and T.G. Puspitasari, 2013. Pembuatan bioetanol dari limbah tongkol jagung dengan variasi konsentrasi asam klorida dan waktu fermentasi. J. Teknik Kimia, 19: 60-69.
3. Bujanovic, B., S. Ralph, R. Reiner, K. Hirth and R. Atalla, 2010. Polyoxometalates in oxidative delignification of chemical pulps: Effect on lignin. Materials, 3: 1888-1903.
4. Perez, J., J. Munoz-Dorado, T. de la Rubia and J. Martinez, 2002. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: An overview. Int. Microbiol., 5: 53-63.
5. Winugroho, M. and S. Marijati, 2001. Konsistesi keefektifan bioplus serat selama masa simpan pada suhu ruang [Consistency of fibre bioplus effectiveness during storage period at room temperature]. Proceedings of the Seminar Nasional Teknologi Peternakan Dan Veteriner, September 17-18, 2001, Balai Penelitian Ternak, Bogor, pp: 214-218.
6. Severe, J. and D.R. ZoBell, 2012. Review: Technical aspects for the utilization of small grain straws as feed energy sources for ruminants: Emphasis on beef cattle. April 2012. https://digitalcommons.usu.edu/extension_curall/95/
7. Datta, R., A. Kelkar, D. Baraniya, A. Molaei, A. Moulick, R.S. Meena and P. Formanek, 2017. Enzymatic degradation of lignin in soil: A review. Sustainability, Vol. 9, No. 7. 10.3390/su9071163.
8. Ottosson, E., 2013. Succession of wood-inhabiting fungal communities: Diversity and species interactions during the decomposition of Norway spruce. Ph.D. Thesis, Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden.
9. Hammel, K.E. and D. Cullen, 2008. Role of fungal peroxidases in biological ligninolysis. Curr. Opin. Plant Biol., 11: 349-355.
10. Goering, H.K. and P.J. van Soest, 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures and Some Applications). Agriculture Handbook No. 379, Agriculture Research Service, United States Department of Agriculture, USA., pp: 387-598.
11. Imsya, A., E.B. Laconi, K.G. Wiryawan and Y. Widyastuti, 2014. Biodegradasi lignoselulosa dengan *Phanerochaete chrysosporium* terhadap perubahan nilai gizi pelepah sawit. J. Peternakan Sriwijaya, 3: 12-19.
12. Kymalainen, M., 2015. Moisture sorption properties and fungal degradation of torrefied wood in storage. Ph.D. Thesis, Department of Forest Sciences, Faculty of Agriculture and Forestry, University of Helsinki, Finland.
13. Isroi, R. Millati, S. Syamsiah, C. Niklasson, M.N. Cahyanto, K. Ludquist and M.J. Taherzadeh, 2011. Biological pretreatment of lignocelluloses with white-rot fungi and its applications: A review. BioResources, 6: 5224-5259.
14. Sukumaran, R.K., R.R. Singhania and A. Pandey, 2005. Microbial cellulases-production, applications and challenges. J. Scient. Ind. Res., 64: 832-844.
15. Chang, S.T. and P.G. Miles, 2004. Mushrooms: Cultivation, Nutritional Value, Medicinal Effect and Environmental Effect. 2nd Edn., CRC Press, USA., ISBN: 9780203492086, pp: 451.
16. Fitria, 2008. Pengolahan biomassa berlignoselulosa secara enzimatis dalam pembuatan pulp: Studi kepustakaan [Enzimatic Processing of *Lignocellulosic biomass* for pulp making for pulp making: A review]. J. Teknologi Pertanian, 9: 69-74.
17. Palmer, J.M. and C.S. Evans, 1983. The enzymic degradation of lignin by white-rot fungi. Phil. Trans. R. Soc. Land. B, 300: 293-303.