MODIFICATION OF WHEAT STRAW LIGNIN BY SOLID STATE FERMENTATION WITH WHITE-ROT FUNGI

Maria J. Dinis [a], Rui M. F. Bezerra [b], Fernando Nunes [c], Albino A. Dias [b], Cristina V. Guedes [a], Luís M. M. Ferreira [a], John W. Cone [c], Guilhermina S. M. Marques [d], Ana R. N. Barros [c], Miguel A. M. Rodrigues [a,]

[a]*CECAV- Universidade de Trás-os-Montes e Alto Douro, Department of Animal Science, Apartado 1013, 5001-801 Vila Real, Portugal;* [b]*CITAB - Universidade de Trás-os-Montes e Alto Douro, Departament of Biological and Environmental Engineering, Apartado 1013, 5001-801 Vila Real, Portugal;* [c]*Animal Nutrition Group, Department of Animal Sciences, Wageningen University, The Netherlands;* [d]*Universidade de Trás-os-Montes e Alto Douro, Department of Agronomy, Apartado 1013, 5001-801 Vila Real, Portugal.*

Abstract

The potential of crude enzyme extracts, obtained from solid-state cultivation of four white-rot fungi (*Trametes versicolor*, *Bjerkandera adusta*, *Ganoderma applanatum* and *Phlebia rufa*), was exploited to modify wheat straw cell wall. At different fermentation times, manganese-dependent peroxidase (MnP), lignin peroxidase (LiP), laccase, carboxymethylcellulase (CMCase), avicelase, xylanase and feruloyl esterase activities were screened and the content of lignin as well as hydroxycinnamic acids in fermented straw were determined. All fungi secreted feruloyl esterase while LiP was only detected in crude extracts from *B. adusta*. Since no significant differences (P>0.05) were observed in remaining lignin content of fermented straw, LiP activity was not a limiting factor of enzymatic lignin removal process. The levels of esterified hydroxycinnamic acids degradation were considerably higher than previous reports with lignocellulosic biomass. The data show that *P. rufa*, may be considered for more specific studies as higher ferulic and *p*-coumaric acids degradation was observed for earlier incubation times.

Introduction

Wheat straw is one of the most abundant crop residues in the world and this huge amount of residues may constitute a promising raw material that could potentially be transformed into a more edible feed for ruminants (Rodrigues et al., 2008) or alternatively it could also be used for the production of ethanol (Fang et al., 2002). In either of these possibilities the main constraint to improve hydrolysis of this lignocellulosic material is the complexity of the cell wall structure. The utilization of white-rot fungi enzyme complexes may be considered an alternative research field to increase the accessibility of cell wall structure. The aim of the present work was to (i) study the production of enzyme complexes by four different fungi - *Trametes versicolor* (TV), *Bjerkandera adusta* (BA), *Ganoderma applanatum* (GA) and *Phlebia rufa* (PR) - at different times of incubation under solid state fermentation (SSF) of wheat straw; (ii) to evaluate its influence in the degradation of esterified hydroxycinnamic acids and (iii) in the removal of lignin, the most recalcitrant biopolymer of plant cell wall.

Materials and methods

Four fungal strains were used to produce the enzymatic extracts, *Trametes versicolor*, *Bjerkandera adusta*, *Ganoderma applanatum* and *Phlebia rufa*. Enzymatic extracts were obtained from a solid culture medium containing 15 g of wheat straw with 0.05 g of glucose in 45ml of deionized water. Flasks were incubated at 27°C and fermented straw from four flasks of each fungus was harvested every 7 days until 28 days after inoculation. Enzymatic activities were determined at 25°C using a Helios UV-Vis spectrophotometer (Thermo Fischer Scientific, Waltham, MA, USA), lignin content was determined using the method of acetyl bromide soluble lignin (ABSL) described by Fukushima and Hatfield (2001). For the

determination of hydroxycinnamic acids an adaptation of the procedures described by Chien (1992) was used. Results for lignin (g) and hydroxycinnamic acids (mg) are expressed in relation to wheat straw initial weight (15 g) and data were analysed using one-way Anova.

Results

Regarding to the ligninolytic enzymes (Figure 1), our results show a general predominance on the activity of MnP and laccase. While all fungi tested produced avicelase (Figure 2) its activity was very low, comparatively with CMCase. There were no significant differences for days 7, 14 and 21 (Table 1) and the maximum value observed (Figure 2) was on TV on day 28 (0.03 U/ml). Finally, BA and TV showed a variable production for xylanase with no appreciable change over the 28 days of the trial. Feruloyl esterase activity was quite similar (Table 1) for all fungi ($P<0.05$) with the exception of GA.

Phenolic composition of wheat straw cell wall is presented in Table 2. As expected results showed that ferulic and *p*-coumaric acids were the dominant esterified hydroxycinnamic acids and lignin concentrations are within the range of values normally reported for wheat straw.

The effect of SSF in phenolic composition of wheat straw is presented in Table 3.

These results indicate that all the fungi treatments were able to reduce the content of esterified *p*-coumaric and ferulic acids in higher extent than the application of commercial enzymes, probably due to the synergism between the enzyme complexes produced by the fungi as we have mentioned in a previous work (Rodrigues et al., 2008).

The lignin content decrease did not differ widely between the different fungi treatments (Table 3). However there was a significant decrease ($P<0.001$) in the total amount of lignin from day 7 until the end of the incubation period (Table 3) reaching a value of 33%. When analysing the content of wheat straw lignin without any fungal treatment (Table 2) it is possible to see that this decrease is around 43%, indicating that lignin loss was quite low within the first 7 days of incubation (13%).

Conclusions

Our data show that the enzyme complexes produced by fungi seem to exert their effect in the cell wall structure due to the synergism between the different types of enzymes. In fact, the higher degradation of esterified hydroxycinnamic acids in the first 7-14days of incubation, directly related to the xylanase and feruloyl esterase activities during this period, precede a more intensive degradation of the lignin structures. The present study also indicated that the fungi treatments were able to reduce to a considerable extent the content of esterified *p*-coumaric and ferulic acids. Considering that wheat straw is quite recalcitrant and that results from the application of commercial esterases do not normally approach such high values these results are of substantial interest.

References

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rot fungi on chemical composition and *in vitro* digestibility of wheat straw. Animal Feed Science and Technology 141, 326-338.

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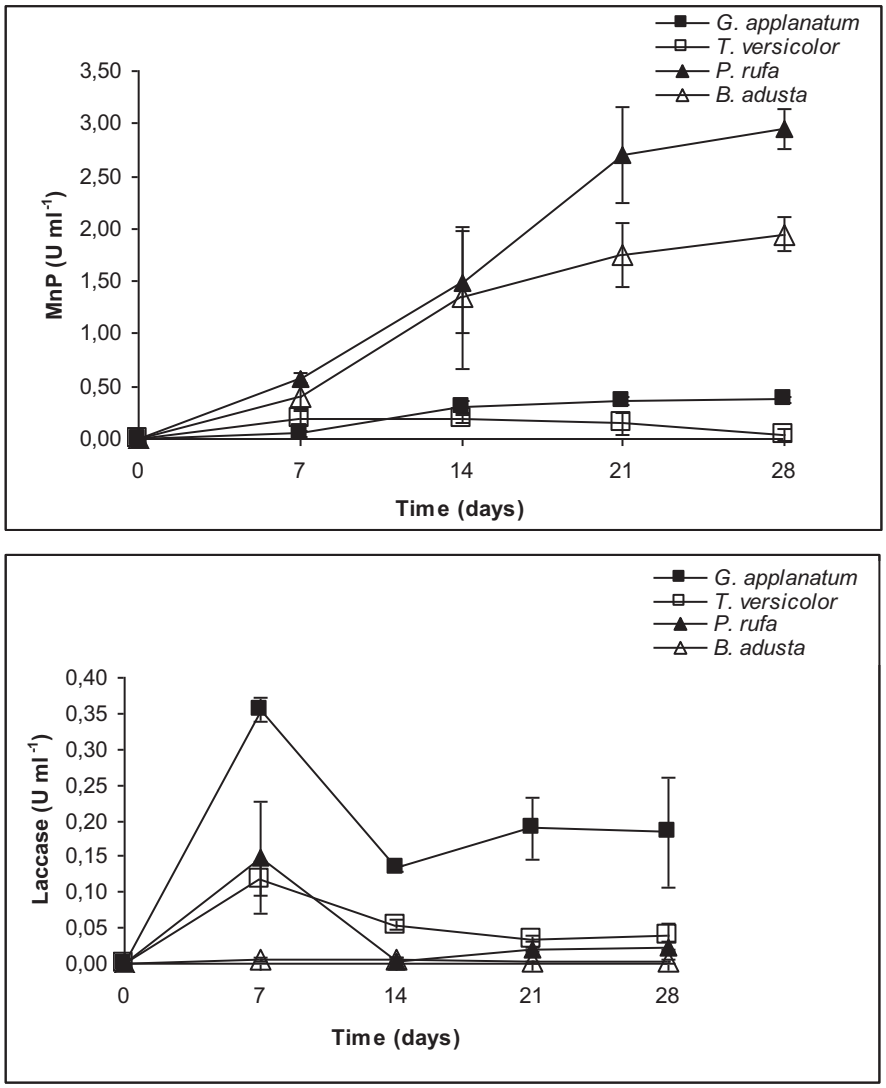
Figure 1 – Ligninolytic enzyme activities of white-rot fungi during the incubation period.



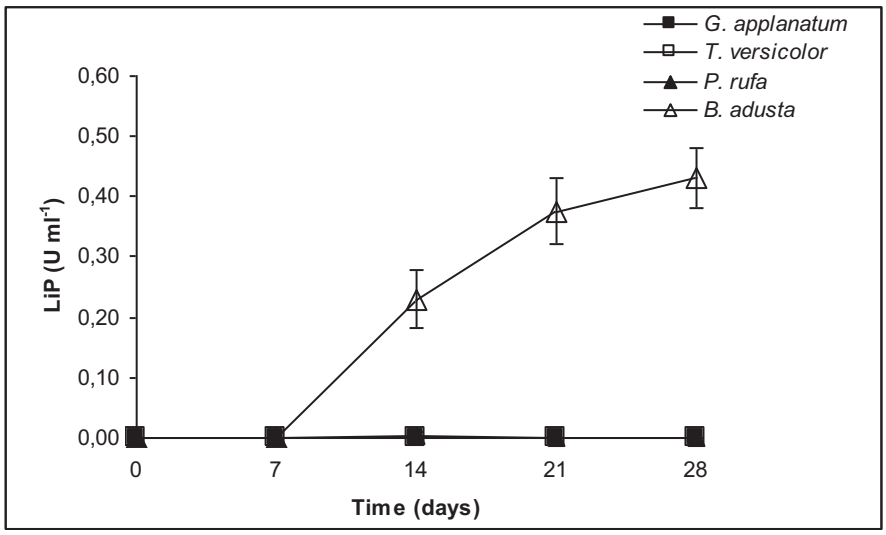
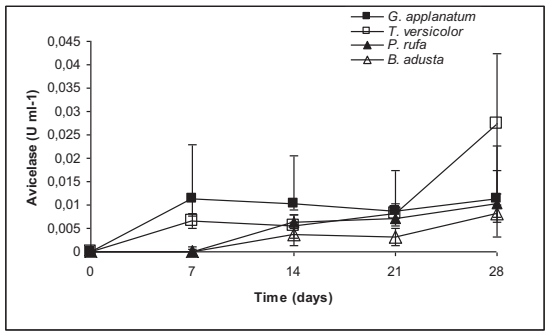
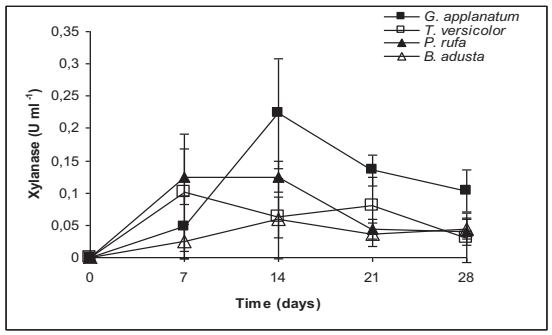
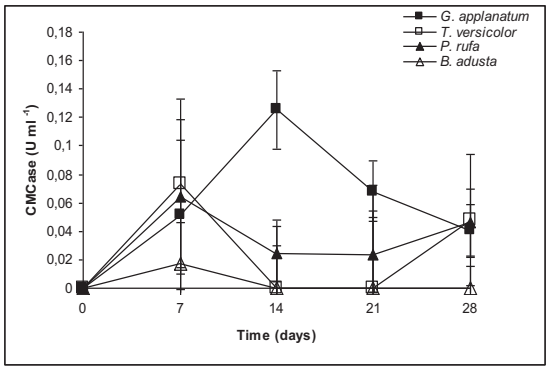


Figure 2 - Cellulolytic, hemicellulolytic and feruloyl esterase enzyme activities of white-rot fungi during the incubation period.







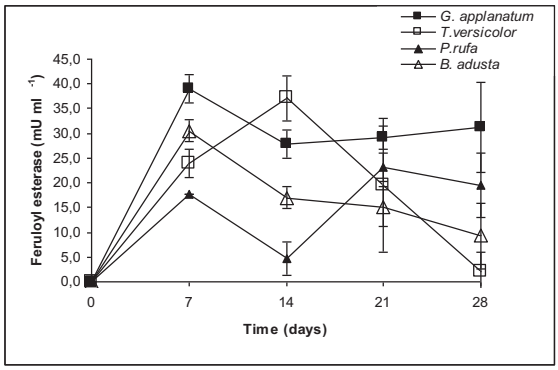
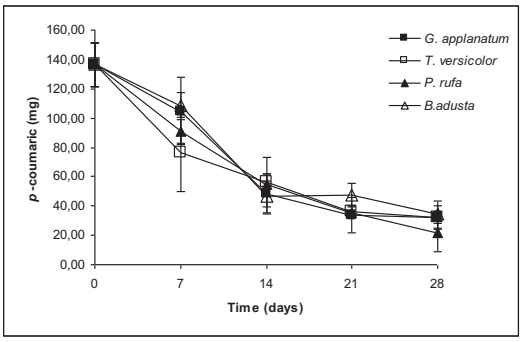
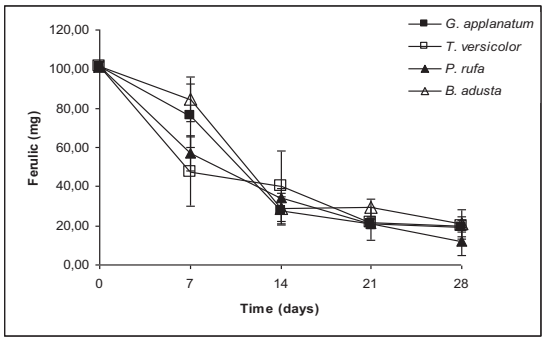


Figure 3 - Time course of hydroxycinnamic acids and lignin degradation during the incubation period.





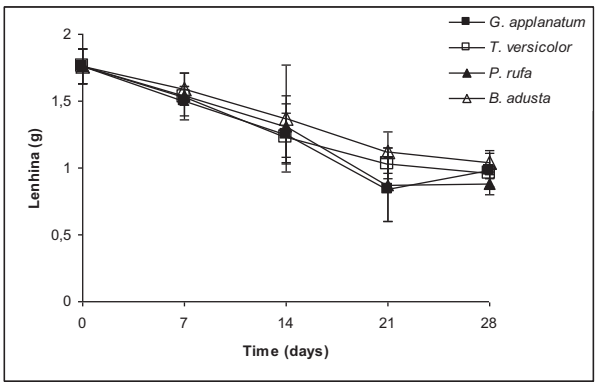


Table 1 - Enzyme activities from four white-rot fungi incubated with wheat straw during 28 days in solid state fermentation.

Source	Enzyme activities (U/ml)						
	MnP	LiP	Laccase	Avicelase	CMCase	Xylanase	Feruloyl
Fungi[A]							
BA	1.363^{b}	0.259^{b}	0.004^{a}	0.006^{a}	0.006^{a}	0.041^{a}	0.018^{ab}
GA	0.271^{a}	0.000^{a}	0.216^{c}	0.003^{a}	0.067^{c}	0.132^{d}	0.032^{c}
PR	1.928^{c}	0.000^{a}	0.049^{b}	0.010^{b}	0.028^{b}	0.063^{b}	0.016^{a}
TV	0.144^{a}	0.000^{a}	0.062^{b}	0.014^{c}	0.054^{c}	0.112^{c}	0.021^{b}
Time (days)							
7	0.307^{a}	0.000^{a}	0.157^{b}	0.008^{a}	0.020^{a}	0.025^{a}	0.027^{c}
14	0.831^{b}	0.058^{b}	0.049^{a}	0.008^{a}	0.016^{a}	0.106^{b}	0.022^{b}
21	1.242^{c}	0.094^{c}	0.061^{a}	0.007^{a}	0.054^{b}	0.109^{b}	0.022^{b}
28	1.326^{c}	0.108^{c}	0.062^{a}	0.011^{b}	0.065^{a}	0.107^{b}	0.016^{a}
Effects[B]							
Fungi	***	***	***	***	***	***	***
Time	***	***	***	***	***	***	***
Time*Fungi	***	***	***	***	***	***	***

Values within a column bearing the same superscript are not significantly different (P>0.05) according to Tukey's test.

[A] BA, *Bjerkandera adusta*; GA, *Ganoderma applanatum*; PR, *Phlebia rufa*; TV, *Trametes versicolor*.

[B] ** P<0.01; ***P<0.001.

Table 2 - Phenolic composition of wheat straw before incubation.

Phenolic composition [A]				
Esterified hydroycinnamic acids (mg)				Lignin (g)
SyrA	CafA	p-CoumA	FerA	
15.4	3.6	136.3	101.2	1.76

[A] SyrA, Syringic acid; CafA, Caffeic acid; p-CoumA, *p*-coumaric acid; FerA, Ferulic acid.