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The Role of Lignin on Decay Resistance of Ulin (*Eusideroxylon zwageri T.* et *B.*) to Wood-rotting Fungi

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I. Introduction

In the previous papers it is reported that the wood of Ulin (*Eusideroxylon zwageri T.* et *B.*), a species of tropical hardwood, is very resistant to decay, in which Eusiderin, a neolignan and major compound in *n*-hexane soluble fraction of the acetone extract, is considered to be responsible for the resistance of the wood[1, 2]. However, after the acetone extraction, decay resistance of Ulin's woodmeal is not significantly reduced. Having regard to the information above, it is assumed that there might be another factors which affect the resistance of wood attacked by wood-rotting fungi. Lignin is one of the factors which is expected to be responsible for the resistance of the wood, due to the knowledge that the plant cell wall is protected against fungal attack through the process of lignification.

Previous researchers reported that the slow degradation rate of softwood species by white-rot fungi is not due to the inhibitory effect of extractives in wood[3, 4]. Other studies have proposed that the differences in the type and content of lignin between hardwood and softwood, must be considered as a factor which is responsible for the resistance of the wood[3, 5]. The effect of lignin on soft-rot fungus behaviour was reported on the basis of the study on delignified and normal sapwoods of Scotch Pine and Birch[6]. The results indicate that the high lignin content of Scotch pine wood has a definite effect in protecting the wood from soft-rot fungi, and a removal of lignin not only can result in large increase in decay but also influence the mode of action of the fungi. Lignin-degrading ability of white-rot fungi is widely known and has recently been regarding by some researchers as an effective means to gain access to the cellulose in a lignified cell wall[7]. According to this theory, the removal of lignin from cell wall may facilitate the action of cellulolytic enzymes produced by white-rot fungi during the attack of wood[8].

The purpose of this study is to investigate the effect of delignification or lignin content of the wood of Ulin on decay resistance to the wood-rotting fungi.

II. Materials and Methods

A. Preparation of samples

Three samples of woody species like Ulin (*Eusideroxylon zwageri T.* et *B.*), Buna (*Fagus crenata*), and Sugi (*Cryptomeria japonica*) were used in this experiment in the form of 40~60 mesh of wood meals, produced by dry Willey milling. The first one, is a typical tropical hardwood, while the last two species which represented a hardwood (Buna) and a softwood (Sugi) of temperate species were used in this experiment as a comparison.

The wood of Ulin was successively extracted with alcohol-benzene (1 : 2) and alcohol solely, whereas Buna and Sugi were extracted only with alcohol-benzene (1 : 2). These extracted wood meals were further used for lignin determination, delignification, and wood decay test.

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B. Fungi and medium

Two species of a typical wood-rotting fungi were used in this experiment, namely Kawaratake (*Coriolus versicolor*) which belongs to white-rot fungi and Ouzuratake (*Tyromyces polutris*) which belongs to the brown-rot fungi.

The composition of the basal medium for the culture of fungi are 50 gr/*l* glucose, 5 gr /*l* polypepton, 0.3 gr/*l* K_2HPO_4, 0.3 gr/*l* KH_2PO_4, 0.2 gr/*l* $MgSO_4 \cdot 7H_2O$, 30 gr/*l* agar, and 120 gr/*l* onion extract.

C. Determination of lignin properties

The Klason lignin content was determined according to the TAPPI STANDARD T-222 SO 74[9]. To determine the properties of lignin, the procedure of alkaline nitrobenzene oxidation recommended by MESHIZUKA[10] was applied with several modifications.

Approximately 50 mg of extracted wood meals were oxidized with 0.24 m*l* nitrobenzene and 4 m*l* of 2N KOH, in a stainless steel tube for 2 hours, at 160℃. At the end of the oxidation period, the stainless steel tube was immediately cooled in water to stop the reaction, and filtered. The oxidation products which still remain in the residue were then extracted by using 0.1N KOH solution. After removing nitrogenous compounds and insoluble materials from the alkaline solution with chloroform, the pH of this alkaline solution was adjusted to 2.5 with 1N HCl. Finally, the solution was extracted for four times with 30 m*l* of chloroform. This extract was then quantitatively analyzed by using gas chromatography under the following condition: 1% OV-1 glass column with column size 1 m and carrier gas He, flow rate 15 m*l* per minute and temperature 180℃.

D. Delignification

Delignification by chlorite treatment has been carried-out according to AHLGREN and GORING's procedure[11]. The initial chemical charge was 0.3 gram of sodium chlorite and 0.1 m*l* of glacial acetic acid per gram of dry wood meal. The liquor to wood meal ratio was 15 : 1 and the temperature was 70℃. The wood meal was treated for different lengths of times. At hourly intervals, a fresh charge of chemicals were added without withdrawal of any liquor. At the end of the treatment, the residue was filtered and washed with distilled water. Finally, the Klason lignin content of delignified wood meal was determined. The lignin removal was calculated based on the differences of Klason lignin content between untreated and chlorite treated wood meals.

E. Determination of sugars content

Carbohydrates determination has been carried out according to the procedure of BORCHARDT and PIPER[12]. Approximately 0.3 gram of extractives-free wood meal was hydrolyzed with 3.0 m*l* of 72% sulfuric acid for one hour at 30℃. The hydrolyzate was diluted with 84 m*l* of water and heated in a water bath for 6 hours at 100℃. The hydrolyzate was then cooled and exactly 0.100 gram of myoinositol was added to it. Next, the hydrolyzate was neutralized with a saturated solution of barium hydroxide to a pH of 5.5, and centrifuged. About 25 m*l* of clear supernatant solution was transferred to a round bottom flask (75–100 m*l*). About 0.07–0.08 gram of sodium borohydride was added to the flask, mixed, and allowed to react for two hours at room temperature. The excess of borohydride was decomposed by adding acetic acid until gas evolution ceases. Next, the solution was concentrated to a sirup with a rotary evaporator at 60℃. About 10 m*l* of methanol was added to the concentrated solution and evaporated to dryness. This procedure was repeated. It was then dried in an oven at 105℃ for 20 minutes to insure complete removal of the water. The solid matter was then acetylated with a mixture of 7.5

Table 1. Nitrobenzene oxidation products (%)*

	Eusideroxylon zwageri (Ulin wood)	*Fagus crenata* (Buna wood)	*Cryptomeria japonica* *(Sugi wood)*
Vanillin	23.3	7.9	26.1
Syringaldehyde	7.4	25.9	–
p-Hydroxybenzaldehyde	–	–	0.4
Total aldehyde	30.7	33.8	26.5
S/V ratio (molar)	0.26	2.74	–
Klason lignin (%)	29.09	21.68	30.49

* All percentages are based on the Klason lignin content.

m*l* of acetic anhydride and 0.5 m*l* of concentrated sulfuric acid for one hour at 60℃. The flask was removed from the water bath, and cooled. The acetylation mixture was then poured slowly with stirring into about 70 m*l* of a water-ice mixture. The mixture was transferred to a separatory funnel, and extracted with methylene chloride. The methylene chloride extract was then concentrated to dryness with a rotary evaporator. The alditol acetates was next dissolved in 2 m*l* of methylene chloride. About 1 μl of this solution was injected into gas chromatography for analysis, under the following conditions: EES glass column, carrier gas He, flow rate 15 m*l* per minute and temperature 180℃. Observed monosacharides, glucose, arabinose, mannose, galactose, and xylose, were summed up as easily hydrolyzable sugars.

F. Determination of specific gravity

Determination of specific gravity of the samples was carried-out according to the procedure of BROWNING[13].

G. Wood decay test

The samples used in this experiment were normal and chlorite-treated wood meals at different lengths of times. The procedure of this test was essentially described in the previous paper[2].

Ⅲ. Results and Discussion

A. Lignin properties

As given in Table 1, the Klason lignin contents of Ulin, Buna, and Sugi are 29.09%, 21.68%, and 30.49%, respectively. Herein, the lignin content of Ulin is not extremely high. This lignin content is commonly obtained for the tropical hardwood species[23, 24]. In order to get more detailed information on the chemical properties of lignin, alkaline nitrobenzene oxidation was carried-out. The results, given in Table 1, showed that the guaiacyl residue of Ulin is extremely high (23.3%), compared to the lignin of Buna (7.9%), while in comparison with the Sugi (26.1%) the content of guaiacyl residue is almost similar in both species. On the contrary, the syringyl residue of Ulin is very low (7.4%), opposite to the syringyl residue of Buna (25.9%). Based on the above data, it can be concluded that the wood of Ulin primarily constitutes of guaiacyl lignin, notwithstanding the fact that the wood is a typical hardwood. The lignin properties of this wood is similar to that of some tropical hardwoods species such as Ceibo wood (*Erythrina crista-galli*)[14], as well as Bangkirai (*Shorea laevifolia*) and Koki (*Hope pierrei*)[15].

Table 2. Lignin removal by chlorite treatment

Wood samples	Delignification time (hour)	Lignin removal (%)*	Klason lignin (%)**	Easily hydrolyzable sugars (%)**
Ulin	0	0.00***	29.09	35.00
	1	15.61	24.55	33.75
	2	38.29	17.95	25.15
	3	61.60	11.17	21.36
Buna	0	0.00***	21.68	34.84
	1	40.45	12.91	32.71
	2	59.64	8.75	47.83
	3	69.33	6.65	45.63
Sugi	0	0.00***	30.49	31.19
	1	42.97	17.49	27.47
	2	51.31	14.82	25.67
	3	68.82	9.49	25.76

* Based on the Klason lignin content.
** Based on the oven-dry wood.
*** Not treated with chlorite.

B. Delignification

The results of delignification process, given in Table 2, shows that the delignification rate of Ulin is slower than those of Buna and Sugi. Surprisingly, after one hour of delignification, the lignin removal from Ulin is 15.61%, extremely different from those of Buna and Sugi where the lignin removal are 40.45% and 42.29%. Table 2 also showed that the increase in lengths of delignification time caused the increase in lignin removal of the three samples. After two hours of delignification, the lignin removal from Ulin, Buna, and Sugi are 38.29%, 59.64%, and 51.31%, respectively. After three hours, the lignin removal of the three wood samples are nearly equal. At the same time, there is also a change in the carbohydrates content of the chlorite-treated samples. The change in the carbohydrates contents were determined by measuring the content of hydrolyzable sugars of untreated and chlorite-treated wood meals, which were hydrolyzed with 72% sulfuric acid. Sugars (hydrolyzable sugars), which were hydrolyzed with 72% sulfuric acid, were tentatively evaluated as an indication of nutrient sources for the growth of fungi. The hydrolyzable sugars content of untreatd and chlorite-treated wood meals is given in Table 3. This table shows that excepted for Buna wood meal, there is decrease in the hydrolyzable sugars content of the chlorite-treated Ulin and Sugi wood meals. The hydrolyzable sugars content of untreated Ulin and Sugi wood meals are 35.00% and 31.19%. After three hours of treatment, the hydrolyzable sugars content of these wood meals are 21.36% and 25.76%, respectively.

The slow delignification rate of Ulin wood meal might be due to a complex structure of associated high polymers, which is indirectly related to the wood density. Determination of specific gravity of Ulin showed that this wood has an extremely high value of specific gravity (1.04). Usually, a species with a high specific gravity possesses a high wood density, and wood with a low specific gravity have a low wood density. In this case, the wood of Ulin with specific gravity (dense wood) is difficult to be delignified because the available space in the wood cell is limited for the chemicals to penetrate though wood meals used in the delignification experiment in the form of 40–60 mesh.

Table 3. Weight loss of normal and delignified wood attacked by wood-rotting fungi after four months of incubation

Wood samples	Lignin content (%)	Easily hydrolyzable sugars (%)	*Coriolus versicolor* (%)*	*Tyromeces polutris* (%)*
Ulin	29.09**	35.00**	2.41	4.50
	24.55	23.75	27.73	40.60
	17.95	25.15	29.01	52.91
	11.17	21.36	34.57	55.29
Buna	21.68**	34.84**	48.48	61.07
	12.91	32.71	53.36	79.36
	8.75	47.83	61.00	80.27
	6.65	45.63	62.39	78.95
Sugi	30.49**	31.19**	11.33	16.83
	17.36	27.47	40.62	47.63
	14.82	25.67	38.59	48.02
	9.49	25.76	39.51	48.45

* Weight loss by decay, percentages based on the oven-dried wood.
** Not treated with chlorite.

C. Weight loss by decay

In order to get a better understanding on the influence of the lignin content on fungal attack on wood, a decay experiment of delignified wood meals was conducted. The lignin contents of wood meals used in this experiment were set at a various different level by partial delignification using chlorite treatment, as described previously. The results of this experiment is given in Table 3. Without delignification, the percentages of weight loss of Ulin, Buna, Sugi decayed by *Coriolus versicolor* are 2.41%, 48.48%, and 11.33%, respectively. Under the same condition, the percentages of weight loss of these wood samples decayed by *Tyromyces polutris* are 4.50%, 61.07%, and 16.83%, respectively.

The results also show great differences in the decay capacity of fungi in undelignified and delignified wood of Ulin. The weight loss, decayed by *C. versicolor* and *T. polutris,* in the one hour chlorite-treated wood meals reached almost 27.73% and 40.60% after four months incubation, while it was only 2.41% and 4.50% in untreated wood meals. When the lignin content of Ulin was lowered from 24.55% to 17.95%, the percentages of the weight loss does not significantly increased, its weight loss only 29.01% and 52.91%. Likewise, as the lignin content was reduced to 11.17%, the increase in the weight loss continue only up to 34.57% and 55.29%. The same pattern was found for Buna and Sugi, where the reducing of lignin content is not closely correlated to the increase in the weight loss after fungal attack.

From this results, it can be stated that delignified wood meals is more readily degraded than undelignified wood meals. But, a strong correlation between the degree of lignin removal by chlorite treatment and the change in the weight loss by fungal attack has not been found in the present study. Based on the above results, it is suggested that the loss of some carbohydrates content during delignification process might be correlated to the increase in the weight loss of partial-delignified wood meals. The correlation between the hydrolyzable sugars content and the weight loss of wood meals was observed, as seen in Table 3. This table shows that except for Buna wood meal, reducing of hydrolyzable sugars content in the partial-delignified wood meals is followed by the increase in the weight loss

by fungal attack. These data, however, showed an unexpected results. So the correlation between the hydrolyzable sugars content and the weight loss of wood meals by fungal attack still remains unclear. But, some researchers suggested that the loss of hemicellulose during delignification process can result in increase in decay capacity of fungi, such as the following results.

Many investigators[16, 17, 18] drew attention to the effect of a delignification process on the hemicellulose as a structural component of the wood cell walls. They suggested that some of the hemicelluloses was lost during the delignification process. Another researcher[19] has proposed that the cellulose cannot be degraded unless the xylan is partly removed from the wood cell wall. Here, delignification could be interpreted as being responsible for removal of hemicellulose which then exposes to cellulose and increases the opportunity for cellulose degradation. From the results of the previous investigators, it is more likely that hemicellulose plays an important role in the partial-delignified wood decay.

Table 3 also showed a great difference in the weight loss of untreated Ulin and Buna wood meals, decayed by the two fungi tested. The differences in the weight loss of untreated Ulin and Buna wood meals might be explained by the different chemical properties of the samples. Studies on the role of lignin structure on decay resistance of wood to the wood-rotting fungi has been carried-out by many researchers. They suggested that wood containing guaiacyl-rich lignin is more resistant to the wood-decaying fungi than that containing syringylrich one[20, 21, 22]. As mentioned above, the lignin of Ulin wood is primarily constituted by guaiacyl lignin, notwithstanding to the fact that the wood is a typical hardwood one. The results of a study on the role of lignin structure of Ulin wood on decay resistance to the white-rot fungus of *Coriolus versicolor* will be reported (now in preparation).

Summary

The effect of delignification on decay resistance of Ulin (*Eusideroxylon zwageri T.* et *B.*) to the fungi of *Coriolus versicolor* and *Tyromyces polutris* was studied. The preliminary study of the lignin properties of Ulin revealed that the lignin of this wood primarily constitutes of guaiacyl lignin, notwithstanding the fact that the wood is a typical hardwood. The results of delignification process showed that at one hour delignification by chlorite treatment, the delignification rate of Ulin is extremely slower than those of Buna (*Fagus crenata*) and Sugi (*Cryptomeria japonica*). In order to get more information about the influence of lignin on the fungal attack on wood, a decay experiment on normal and delignified wood meals was conducted. The wood decay test showed that there is great differences in the weight loss of normal and delignified wood of Ulin after being exposed to the fungi for four months of incubation. The result of the present investigation indicated that the degree of lignin removal from wood by chlorite treatment is not closely correlated to the change in the weight loss after fungal attack.

Key words: Ulin wood (*Eusideroxylon zwageri*), Delignification, Decay, Durability, Basidiomycetes

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ウリン材の耐朽性におけるリグニンの役割

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要　旨

ウリン材の大きな耐朽性の一因がリグニンにあると考えてその性状をブナ，スギと比べながら追求した．リグニン量はスギなみであったが，廣葉樹材であるのにグアイアシル基が過半をしめた。また亜塩素酸塩処理をしたときのリグニンの除かれ方がブナ，スギより著しく遅かった。

0, 1, 2, 3 時間亜塩素酸塩処理した木粉夫々でカワラタケ，オオウズラタケを培養して木粉の重量減少を調べた。処理時間の長い木粉ほどリグニン含量は小さく，腐朽による重量減少は大きかったが，両者の間に強い相関は認められなかった。

キーワード：ウリン材，脱リグニン，腐朽，耐朽性，担子菌