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CHEMICAL CHARACTERISTICS OF *EUCALYPTUS PELLITA* WITH HEART ROT

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ABSTRACT

Eucalyptus pellita has been posited as a primary raw material in Indonesia due to its fast growth. In some areas, however, trees with heart rot were found. Thus, the wood with heart rot was analysed chemically both in sound (sapwood, outer heartwood, inner heartwood) and degraded parts (heart rot-affected wood/HRAW). The results revealed that there was a different trend in the wood chemical composition between bottom and centre parts. In bottom parts, wood with bigger diameter of heart rot, the slight changes in polysaccharides and lignin amounts was observed in HRAW compared to sound wood parts. On the contrary, comparatively high lignin and low polysaccharide levels in HRAW were measured in centre parts. HRAW was also characterized with high content of inorganic materials and high pH values but low in extractive content, mostly ethanol soluble extractives or its polar fraction. Increasing of phenolic contents was more pronounced in HRAW of lower part than that of upper of the stem. The difference trend of chemical composition between bottom and centre parts suggesting the cause of heart rot could be several wood degraders.

KEYWORDS : Heart rot, cell wall, wood chemistry, phenolic, extractives, *Eucalyptus pellita*.

INTRODUCTION

To keep up with the increasing demand for pulp and paper, *Eucalyptus pellita* F. Muell or Red mahogany has been extensively planted for pulpwood production in Indonesia started from 1994. The desirable characteristics of *E. pellita* include fast growing and a high resistance to diseases and pests so that several tree breeding programs have targeted this species (Leksono et al. 2008). Subsequently, the potential as pulpwood of *E. pellita* grown in Indonesia has been studied in terms of basic properties (Susilawati and Marsoem 2006, Susilawati and Fujisawa 2002, Fatimah et al. 2013, Lukmandaru et al. 2016).

As in *Acacia mangium* wood, however, *E. pellita* trees with heart rot were also found in some areas. This is a major problem as the occurrence would reduce the utilization of the timber for final products. In our best knowledge, no study have been conducted to explore the frequency of this phenomenon nor the causes and the characteristics of *E. pellita* wood with heart rot. With regard to *A. mangium* wood, extractive composition as well as the phenolics of the heartrot-affected wood has been investigated (Barry et al. 2005, Lange and Hashim, 2001, Mihara et al. 2005). Thus, this work aimed to examine the composition of cell wall components as well as the extractives of *E. pellita* wood with heart rot. Analysis of heart-rotted wood is valuable to predict the potential causing-organisms as well as to find out the how much the differences between the healthy and attacked tissues for timber utilization.

MATERIALS AND METHODS

Sample materials

Wood samples were obtained from a single tree (dbh of 55 cm, 36 years) grown at the campus yard of Faculty of Forestry, Universitas Gadjah Mada, Jogjakarta. The 5-cm-thick discs were collected at 30 cm from the base part (bottom) and center part of the tree. This tree showed a huge diameter (± 35 cm) of heart rot in the bottom part and smaller diameter (± 5 cm) in the centre part.

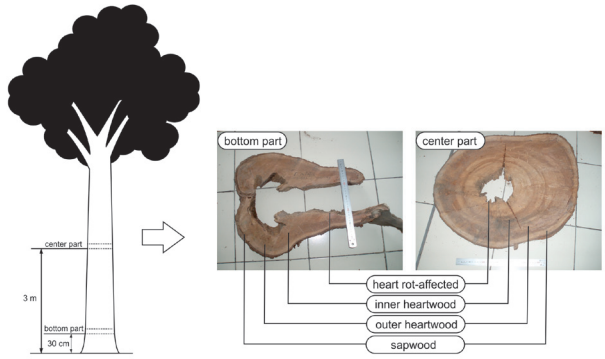

Fig. 1: Scheme of wood sampling of heart rot-affected of a E. pellita tree.

The test specimens were taken successively from sapwood to heartwood, and divided into four sections diametrically (Fig. 1) i.e. sapwood (SW, ca. 0.5 cm from the bark), outer heartwood (OH, ca. 0.5 cm from the heartwood-sapwood boundary), inner heartwood (IH, ca. 3 cm from the innermost) and heart rot-affected wood (HRAW, thickness of ca. 1.5 cm).

The HRAW parts were marked by softer and darker wood than the adjacent normal tissues.

Cell wall components and extractives determination

Lignin content, ash content, solubility values in 1% NaOH, hot water, ethanol 95%, and alcohol-toluene were determined according to ASTM standard methods (2002). The extractions by ethanol-toluene, and hot water were conducted separately. Cellulose and holocellulose content were determined according to Wise's chlorite method (Browning, 1967), while acid insoluble ash content was according to TAPPI T 244 cm- 99 (1999). Hemicellulose contents were calculated by subtracting holocellulose from the cellulose contents.

Successive extraction was conducted in 5 g oven-dry wood meals by dichloromethane, ethanol, and hot-water in a sequence. Extractions by dichloromethane and ethanol were carried out in a soxhlett apparatus for 6 hours. Hot-water extraction was performed by refluxing in a water bath for 3 hours. The solvents were concentrated in a rotary film evaporator, dried, and weighed to determine the dichloromethane (DEC), ethanol (EEC), and hot-water (HWC) extractive content based on oven dry wood meal (m/m). The total extractive content (TOEC) was calculated by determining the sum of all extractive contents.

Spectrophotometric analysis

Total phenolic, flavonoid, and flavanol contents were determined colorimetrically with a Nano 3000SP UV-Vis spectrophotometer in extracts obtained by ethanol extraction. The analyses were done in triplicate . The results were expressed as a mean value.

The total phenolic content (TPC) was conducted by the Folin-Ciocalteu method (Gao et al. 2006) as calibration was achieved with gallic acid aqueous solution. Further, total flavonoid content (TFC) was calculated by using the AlCl3 method (Diouf et al. 2009) and using quercetin as standard solution. Total flavanol content (TVOC) was determined by vanillin assay as described by Scalbert et al. (1989). Results were expressed in (+) –catechin equivalents per mass of dry wood.

pH value measurement

Measurement of acidity of the wood was determined by its pH value. Wood powder (1 g oven-dry wood meal) was soaked for 28 hours in distilled water (20 mL). The pH of the filtrate was measured with a pH meter (OAKTON pH tester). Three measurements were made for each part.

RESULTS

The contents of cell wall component, inorganic materials, and pH values in the different radial position are presented in Tab. 1. It was notable that from outside to inside, the content of lignin and solubility in 1% NaOH was the highest whereas holocellulose and α-cellulose contents were the lowest in OH of bottom part of the tree. A different trend was found for centre part of the tree, where the highest levels of holocellulose, α-cellulose, hemicelluloses and the lowest level of lignin and solubility in 1% NaOH were measured in SW.

In the bottom part, HRAW was marked by considerably greater amounts of inorganic materials as well as pH values compared to the normal tissues as no systematic differences was found in the amount of cell wall components. A different trend was observed for centre part; HRAW displayed drastically acccumulation of lignin, solubility in 1% NaOH, inorganic materials, and pH values but reduced in holocellulose, α-cellulose and hemicellulose contents than those of sound parts. HRAW in bottom part analysed showed a different composition compared to centre part; hollocelulose, and α-cellulose contents were accumulated in a greater degree while lignin, solubility in 1% NaOH, inorganic materials, and pH values were reduced.

Tab. 1: The contents of cell wall component, inorganic materials, and pH values of E. pellita wood with heart rot.

Position	Lignin (%)[a]	Holocellulose (%)[a]	α-cellulose (%)[a]	Hemicellulose (%)[a]	1% NaOH (%)[b]	Ash (%)[b]	Acid insoluble ash (ppm)[b]	pH value
Bottom sapwood	31.07	72.04	38.47	33.57	28.08	0.38	0.90	4.68
outer heartwood	35.81	65.71	35.37	30.34	36.57	0.26	0.15	4.19
inner heartwood	32.72	69.58	42.67	26.91	25.66	0.05	0.40	4.50
heartrot - affected	32.25	69.02	42.52	26.50	23.01	4.09	32.80	5.05
Centre sapwood	30.71	69.84	37.05	32.79	26.64	0.52	0.55	5.37
outer heartwood	31.98	66.31	35.67	30.64	39.73	0.22	0.65	4.21
inner heartwood	32.64	67.68	36.72	30.96	33.25	0.15	0.85	4.30
heartrot - affected	45.80	48.94	23.55	25.39	60.65	9.74	76.00	5.50

Note : a = based on extractive-free wood meal; b = based on oven-dry wood meal

The extractives of *E. pellita* wood were dissolved separately by ethanol-toluene and hot water. By this procedure, yields are given in Tab. 2. Compared to sound wood, there was a strong decrease in ethanol-toluene soluble content (ETC) and hot-water solubility (HWC) levels in HRAW of bottom part. A similar trend was observed in center part, which ETC level in HRAW was considerably lower than undegraded wood except for SW. On the contrary, HWC of HRAW in centre part was relatively high. Further, it was also noticed that the highest ETC value was unusually observed in SW of bottom part although it showed the lowest value in SW of centre part. Comparison between HRAW of bottom and centre parts showed a remarkably high ETC and HWC levels in the centre parts.

The *E. pellita* wood was also extracted in succession to yield three fractions (Tab. 2). Samples were investigated in different regions, from outside to inside, HRAW of bottom parts showed the highest in dichloromethane soluble extractive content (DEC) and the lowest in ethanol soluble extractive content (EEC) and total extractive content (TOC). Further, in centre parts, HRAW showed the lowest values in EEC and TOC but highest values in HWC. The difference between HRAW of bottom and centre parts was comparatively high of DEC and low amounts of EEC and TOC in bottom parts.

Tab. 2. Extractive content of E. pellita wood with heart rot (based on oven dry wood meal).

Position	Ethanol-toluene (%)	Hot-water soluble (%)	Successive extraction			
			Dichloromethane (%)	Ethanol (%)	Hot water (%)	Total (%)
Bottom sapwood	8.09	2.29	0.36	16.74	2.04	19.14
outer heartwood	7.91	3.39	0.56	19.70	3.40	23.66
inner heartwood	4.65	3.26	0.78	9.40	1.92	12.10
heartrot -affected	2.51	0.90	1.02	3.98	2.76	7.76
Centre sapwood	1.89	3.10	0.26	8.82	2.26	11.34
outer heartwood	14.69	3.85	0.84	25.44	2.12	28.40
inner heartwood	8.38	2.82	0.44	16.86	2.10	19.40
heartrot -affected	4.22	5.55	0.58	6.30	3.39	10.27

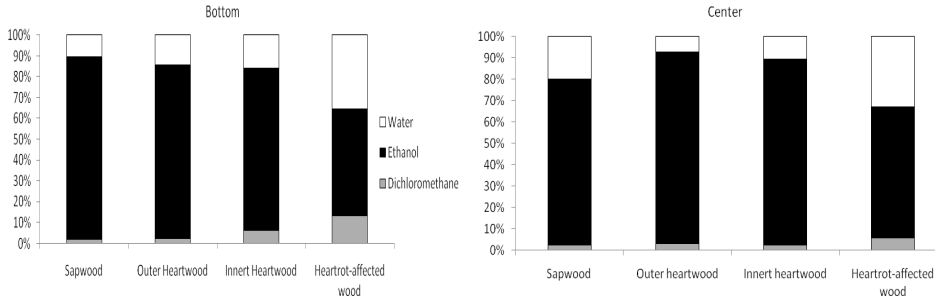

Fig. 2: Composition of extracts by successive extraction in the E. pellita wood with heart rot (based on extract weight).

From the extractive composition (Fig. 2), it revealed that HRAW composed of higher HWC and DEC amounts but lower EEC amounts compared to normal wood both in bottom and centre parts. Differences between the extractives of HRAW of lower and upper part of the tree can be observed by an increase of the EEC and a decrease of DEC and HWC values in upper parts.

Tab. 3. Phenolic contents of E. pellita wood with heartrot (based on ethanol extracts).

Position	Total phenolic content (%)[a]	Total flavonoid content (%)[a]	Total flavanol content (%)[a]
Bottom sapwood	1.58	25.34	7.22
outer heartwood	1.32	32.97	2.00
inner heartwood	1.32	54.98	2.33
heartrot -affected	1.71	86.93	38.50
Centre sapwood	1.27	14.98	12.38
outer heartwood	1.40	25.87	10.64
inner heartwood	1.20	40.4	2.23
heartrot -affected	1.05	70.49	8.12

Measurement of phenolic compounds is presented in Tab. 3. It was observed that the quantity of total phenolic content (TPC) and total flavanol content (TVC) was unusually higher in SW compared to IH and OH of bottom part. For centere part, SW possessed the lowest value of total flavanoids content (TFC) as OH had the lowest value of TVC. The HRAW of bottom part had concentrations of TPC, TFC, and TVC that were higher than normal wood. The HRAW of centre part contained the most TFC but lowest TPC. Compared to centre part, HRAW of bottom part showed higher levels in all phenolic parameters, particularly in TVC.

DISCUSSION

In earlier works, it was reported that fungal decay caused heart rot in *Acacia mangium* (Lee et al. 1988) and *Eucalyptus saligna* (Westhuizen 1959). Further, several white rot and white pocket rot species had been isolated from discoloured wood of *Eucalyptus diversicolor* (Davison and Tay 2008). Heart rot degradation may start already in the heartwood in the living trees. This degradation will change in the proportions of the main components in the remaining wood material. Thus, the wood with heart rot was analysed chemically both in sound and degraded parts. In this experiment, the bigger diameter of heart rot in bottom part of the tree could be related to advanced attacks from degrading-organisms along the time than that in centre part of the tree.

With regard to cell wall components, the most remarkable feature was the different trend between lower and upper parts. Thus, it is suggested that the cause of heart rot could be a combination of some destroying-organisms. The appreciable increase in the relative amount of lignin and solubility in 1% NaOH along with the decrease of holocellulose and α-cellulose contents was only observed in centre part. The changes in chemical composition in centre part is similar to wood attacked by brown-rot fungi which wood polysaccharides degraded in a large extent while lignin is modified or slightly depolymerised (Irbe et al. 2006, Jin et al. 1990). In general, brown rot decay also leads to increased solubility in hot water and in alkaline solutions (Nillson 2009). The slight changes in polysaccharides and lignin content in bottom part was possibly due to white rots. It is known that a typical feature of certain white rot results in a rather uniform losses of cellulose, hemicelluloses and lignin where the proportions among these components remain relatively constant (Nilsson 2009).

The amount of ash as well as silica and silicate indicated by acid insoluble ash content in heart rot wood considerably increased in both bottom and centre parts. It could be due to precipitation of minerals or entering of surrounding soil particles in the wood. Another possibility is due to rot

fungus of white rot as it is capable of redistributing inorganic materials from outside sources to the wood (Nillson 2009). The increased of pH values of degraded tissues seem to be associated with the increased of ash contents. The slightly higher pH values in the radial section were also observed in wood attacked by fungi (Nagadesi et al. 2013, Starck et al. 1984, Tanaka et al. 1986) or termites (Lukmandaru et al. 2015).

Previous studies demonstrated that the role of extractives for protective function ranges from their mechanical barrier (Vek et al. 2013), hygroscopicity (Venäläinen et al. 2004), toxicity, and antioxidative natures (Mihara et al., 2005). In terms of extract yields per unit weight of wood, the amount of ETC and HWC in HRAW was considerably low, particularly in bottom part, suggesting that the attacking organisms were able to consume and degrade extractives to a large extent. On the contrary, the increase in levels of HWC in HRAW of centre part was interpreted as a result of hydrolysis of parts of the polysaccharides as shown in Tab. 1.

By successive extraction, it was found that EEC composed the highest proportion in all radial parts. Therefore, the low amount of TEC in HRAW was mainly due to reduction of EEC. This polar fraction was assumed to be composed predominantly by phenolics. It was noticed that the comparatively high lipophilic content as indicated by DEC in HRAW of bottom part. This result was not in accordance with the findings of previous work (Lange and Hashim 2001) demonstrating that heart rot affected heartwood contain much higher polar extractive fractions than heart rot unaffected samples in *Acacia mangium*. It has been reported that several white rot fungi can reduce extractive content (Martínez-Iñigo 1999, Nagadesi et al. 2013, Starck et al. 1984).

The high content of certain flavonoids which contribute to heart rot resistance as well as their antifungal effects on the Acacia woods was previously investigated (Mihara et al. 2005, Barry et al. 2005). Thus, it was assumed that the tree initially had relatively low amounts of phenolics in near-pith region. In this experiment, the appreciable amount of TFC and TVC in HRAW of bottom parts of the stem could be related to respond against wood degraders for years. On the contrary, the tendency in HRAW in the centre part of the stem suggesting that the phenolics were not fully formed after the rotting process with the exception of certain flavonoids indicated by comparatively high TFC level. The total amount of extractives in heartwood is generally much higher than in sapwood. The unusual high levels of phenolic contents as well as extractive contents in the sapwood were interpreted as the protective role of this tissue which is important for cambium survival after the attacks. This position has previously been put forward in a study of wound-associated wood of beech (Vek et al. 2013). Unfortunately, no phenolic in *E. pellita* wood was reported so far to our best knowledge. Therefore, future works are necessary to explore the role of phenolics in a larger tree samples as well as microscopic analysis to find the cause of heart rot.

CONCLUSIONS

The wood of *Eucalyptus pellita* with heart rot was studied. Compared to sound wood, heart rot-affected wood (HRAW) possessed high amounts of inorganic material and pH value and low amounts of ethanol-toluene soluble extractives. In bottom part, the more severely degraded part, HRAW showed a similar chemical composition of cell wall components to normal tissues. In centre parts, however, changes in chemical composition occurred; polysaccharides were extensively degraded whereas lignin was accumulated. HRAW had high levels of pH values, ash and acid insoluble ash contents, but low amounts of extractive content, mostly for ethanol

soluble fraction. It was found high phenolic contents, particularly in lower parts of the stem. While larger samples are necessary to be analyzed to determine variability between different trees, this preliminary information supports the suggestion that the cause of heart rot could be a combination of some destroying-organisms.

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