Chapter 4

Characterization of black streaked heartwood in teak

4.1. INTRODUCTION

Discolorations in timber and lumber belong to the most serious defects in wood of numerous economically important species from tropical regions. Teakwood, harvested mostly from the island of Java, is the most valuable timber in Indonesia. In certain areas, stems with an irregular black streak zone occur and have been locally called "doreng". This blackening is known to occur in a growing tree and only in the heartwood. The streak generally follows the annual ring and frequently extends from outer to center heartwood. Teak widely used for furniture or parquet, black streak discolorations may lead to considerable degradation and economic loss.

It was suggested by forester workers that from their experience, whether teak timber has doreng wood depends on site conditions. Black streaks have been most frequently recorded as occurring in teak in calcareous soil than in the volcanic ash soil of central Java island (Suhaendi 1998). So far, no specific analysis has been made to explain the characteristics and development of black streak in teak. The main purpose of this investigation was to examine the color and chemical characteristics in wood from black streaked and normal trees.

4.2. MATERIAL AND METHOD

4.2.1 Sample preparation

The samples of doreng tree were felled in the Perhutani Plantantion, Randublatung, Central Java province. The site was black calcareous soil, which the black streaked trees frequently occur. The trees were between 35-50-years old, straight-stemmed, and sound. The 5 cm discs were taken at different heights from the butt end of

the trunks. Further, wood meals of discolored trees were taken from the outer heartwood part in opposite radii, then grinded to size 40-60 mesh wood meals. The meals were then combined for color and chemical parameters determination. The variously black streaked disks were grouped into two types by visual inspection and brightness (L*) value measurement: strong (L* < 45, trees no 1 - 6) and thin (L * ≥ 45, trees no 7-13) streaked heartwood. In addition, five disks were obtained from different trees with normal heartwood in the same sites (trees no 14 to 18). The picture of black streaked heartwood and stand characterization was displayed in Fig. 10 and Table 6, respectively.

4.2.2. Color measurement

Wood color was measured on the air-dried wood meals using NF777 spectrocolorimeter (Nippon Denshoku Ind. Co Ltd.) with a diameter opening of 6.0 mm. Illuminant A and tungsten halogen light source was used. Percentage of reflectance data was collected at 20 nm intervals over the visible spectrum (400 – 700 nm). Three measurements were made for each part. The color is represented by the values L* (brightness), a* (redness), b* (yellowness), chroma or saturation, and hue (angle of a color). The hue and chroma values were calculated by using values a* and b*; hue = arctg (b*/a*) in degrees (deg), chroma = $[(a^*)^2 + (b^*)^2]½$.

4.2.3. Measurement of pH value

One g of wood meal per part was extracted overnight in distilled water (20 ml) and then the pH of the filtrate was measured with pH meter (Horiba). Three measurements were made for each part.

4.2.4. Inorganic elements analysis

Samples (0.2 g) were prepared for analysis using a nitric acid-perchloric acid with a 5 : 3 (v/v) digestion procedure. Measurements of potassium (K), calcium (Ca), magnesium (Mg) and iron (Fe) were carried out using a Hitachi Z-5000 atomic absorption

spectrophotometer. The amount of silica was also determined : filter papers from digesting procedure were burned in a muffle furnace at 30 to 400°C for 120 min, then at 400 to 900°C for 120 min, and finally were maintained at 900°C for 120 min. The ashes were dried for measurement of the true weights.

4.2.5. Extractive content determination

Wood meal (2 g) was extracted exhaustively in Soxhlet extractors. Successive extractions were carried out with *n*-hexane, ethyl acetate (EtOAc), and methanol (MeOH) for about 6 h for each. Cold-water and hot-water extractive content determinations were carried out according to ASTM D-1110 standard method. All of the procedures were repeated twice.

4.2.6. GLC and GC-MS analysis

The GLC and GC-MS analysis of the extracts from *n*-hexane, EtOAc, and MeOH (concentration of 100 mg/ml) were described in the chapter 3.

4.2.7. Statistic analysis

Variation of the color and chemical characteristics between the groups were analyzed (GLM procedure) by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (p=0.05). The relationships between the independent variables were studied with a Pearson's correlation analysis. All statistical calculations were conducted using SPSS-Win 10.0.

4.3. RESULTS AND DISCUSSION

4.3.1 Color measurement

Previous studies on teak grown in India (Bhat et al 2005) and Togo (Kokutse et al. 2006), the brightness index ranged 48 to 58. From the brightness measurement (Table 6),

the highest brightness value of black streaked heartwood is 52, whereas the lowest brightness value of normal heartwood is 51. This means that there is teak with dark heartwood within the range of thin streaked heartwood. Fig. 11 showed that the black streak areas give significantly lower values in yellowness, hue and chroma than normal heartwood areas. Significant differences were also detected between the two black streak areas, with the strong streak heartwood having a less yellow and chroma than the thin streak. The black streak areas give higher redness values than the normal areas, however, ANOVA found significant differences for only thin streak and normal heartwood areas due to the high between tree variation. As expected, Pearson's correlation confirmed the brightness values showed strong correlation with yellownes, hue and chrome (Table 7).

4.3.2 Measurement of pH value

Figure 12 shows the gradients of pH value in the radial direction. All pH values were in the weakly acidic range. A comparison of pH values revealed the discolored wood significantly has higher levels than the normal ones but no significantly differences were observed between the strong and thin discolored samples. The pH value in the black streak portion ranged from 5.58 to 7.07 whereas in the normal heartwood ranged from 4.96 to 5.40. The brightness and pH levels were moderately negatively correlated in Pearson's correlation (r = - 0.74, Table 8). This finding indicates that the discoloration of teak wood would be due to a pH change in the wood although still in weakly acidic range. The correlations between pH values with calcium or potassium content were low (data not shown). These differences in weakly acidic range might be involved in the discoloration from normal to black. Furthermore, there is an alternative in that the blackening phenomenon in teak heartwood: phenolic compounds in the heartwood are oxidatively polymerized under a weakly acidic condition. Therefore, it is necessary to find out the cause of higher pH in the black streak regions and correlate it with the blackening process.

For some wood species, discoloration of wood has possibly been influenced by pH value (Sandermann and Rothkamm 1959). This may also be applicable to teak although

this result indicates the discoloration process does not involve a comparatively huge gradient of pH such as in *Cryptomeria japonica* (Takahashi 1996) or in *Pycnanthus angolensis* (Starck et al. 1984).

4.3.3 Inorganic elements analysis

Fig. 13 shows that the Ca of discolored parts significantly higher than the normal parts although no differences between strong and thin black streak parts was found. The values of discolored portion varied from 2190 to 4760 ppm whereas the normal part varied from 1140 to 2500 ppm. K and silica content of discolored give higher values compared to those of normal part, however, ANOVA revealed only significant differences between the thin streak and normal heartwood areas due to fairly large of between tree variation.

The difference seen between normal and black streak heartwood was not recognized in Mg and Fe levels. Iron, which might bring about formation of a complex salt of dark color by the reaction with phenolic substances in heartwood, did not increase characteristically in the streaked heartwood. This result implies that the formation of blackened heartwood is hardly related to these metals. No significant correlation was found between the brightness index and those inorganic materials levels (Table 8). This fact suggests the mobility of calcium ions is higher than other inorganic materials in heartwood, resulting in a high calcium content in this region. The results of this study do not provide much information about the mechanism through which calcium involved the blackening process. Previous reports on other species showed that inorganic elements in wood could be correlated to the blackening process in heartwood (Kubo and Ataka 1998; Minato and Morita 2005).

4.3.4. Extractives content determination

The extractive content determination is presented in Fig. 14. It is generally assumed that the darker heartwood contains more extractives. The *n*-hexane and EtOAc

extractive content between the groups clearly differed and followed the tendency of the brightness index. Thus, it seems reasonable to assume that the increases in less polar extractives content are due to the production of darkened heartwood. The differences between normal and discolored portion in MeOH and hot water extractive content were significant even though there was no significant difference between the strong and thin black streaked heartwood. The black streak areas give higher cold-water extractive content values than the normal areas, however, ANOVA found significant differences for only thin streak and normal heartwood areas. Those results indicated that black streaked heartwood contained higher both polar and apolar compounds than the normal tissues. It is more pronounced in the EtOAc extractive content levels which strong, thin streaks and normal heartwood areas ranged from 4.38 to 7.42 %, 3.51 to 5.60 %, and 0.75 to 2.13 %, respectively. As expected, among those extractive contents, the strongest degree correlation was observed in brightness-EtOAc extractive content (r = -0.94). The high correlation coefficient between EtOAc extractive content and brightness is particularly emphasized in a scatter diagram (Fig. 15). The correlations between the brightness and other extractive contents were moderate and low.

4.3.5. GLC and GC-MS analysis

The identified major components (Fig. 16) were lapachol, tectoquinone, squalene, tectol (Lemos et al. 1999), and desoxylapachol or its isomer (Perry et al. 1991; Windeisen et al. 2003). The GLC analysis showed that tectoquinone content significantly higher in discolored areas than the normal heartwood (Table 7). Tectoquinone content varied from 0.72 to 3.63 % in black streaks part and 0.29 to 0.84 % in normal part. In contrast, squalene was significantly lower in the discolored part compared to the normal heartwood. The squalene content values of discolored portion varied from 0.26 to 1.45 % whereas the normal part varied from 0.23 to 2.88 %. No significant differences between strong and thin streaks in tectoquinone and squalene content. Tectoquinone content recorded in

literatures (Sandermann and Simatupang 1966; Windeisen et al 2003) are varied from 0.3 to 2 % whereas squalene varied from 1 to 2 %. ANOVA showed no differences between discolored and normal tissues in desoxylapachol, palmitic acid, 2-tert-buthyl-anthraquinone and tectol due to the high between tree variation.

In the living tree discolorations are initiated predominantly through wounds, dying branches and roots (Shigo 1976). In teak, some phenolics have already been identified, however, phenolic compounds involved in wood color remain unknown as yet. The role of tectoquinone in natural durability of teak is recognized in several reports (Rudman et al 1958; Sandermann and Simatupang 1966; Haupt et al. 2003). Although a direct bioassay test was not attempted in this study, it is suggested that the blackening processes may be related to some protective functions against biological origin although the mechanism of resistance associated with the discolored tissue has not been determined. This may have much in common with that in *Diospyros kaki,* in which the black portion of its heartwood is more resistant than the adjacent normal heartwood (Noda et al. 2002).

Tectoquinone content negatively moderately correlated with the brightness value whereas squalene moderately positively correlated with the brightness value. The reason for this relationship does not necessarily have to be related to the chemical nature of tectoquinone, but it could also be a specific feature in the responsible compounds that is correlated with the concentration of anthraquinones. It is also unsure for the cause of lower squalene production in the discolored wood and its relationship with degree of blackening. It is thought that tectoquinone has indirect role by forming polymeric compounds responsible for discoloration which it reduced terpenes production such as squalene.

Table 6. Stand description of black streaked and normal heartwood samples

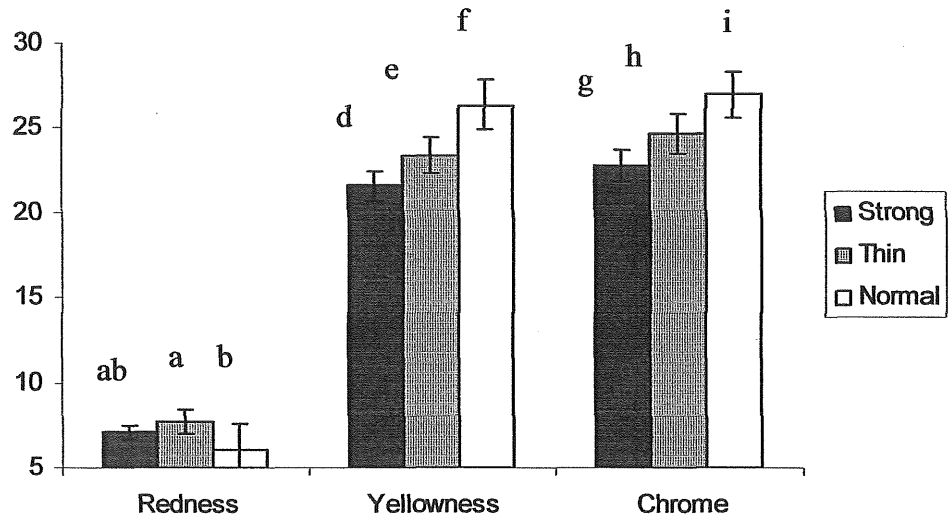
Tree number	DBH age	Diameter (cm)	Brightness (L*)
Strong black streak			
1	35	33.0	40.5
2	29	23.8	41.4
3	29	24.0	42.0
4	28	24.7	43.0
5	37	27.0	44.8
6	37	31.8	44.9
Thin black streak			
7	33	22.1	45.6
8	33	27.2	45.9
9	32	35.2	47.5
10	26	25.6	47.5
11	30	31.8	49.0
12	44	27.3	51.3
13	27	33.5	52.1
Normal			
14	37	39.5	51.7
15	31	32.0	57.7
16	32	26.0	58.6
17	30	23.5	57.3
18	30	25.5	61.3

Table 7. Pearson's correlation coefficients between brightness index (L*) and other independent characteristics.

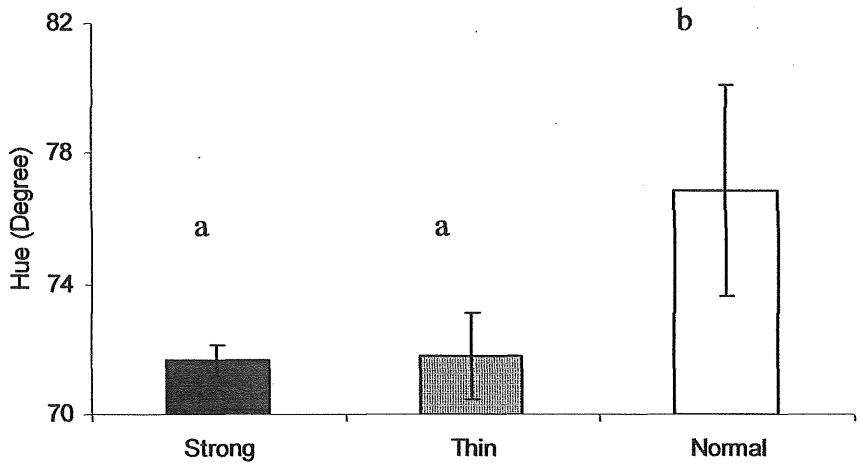
Parameters	Brightness (L*)
Color properties :	
a*	-0.49*
b*	0.92**
hue	0.82**
chrome	0.86**
pH value	-0.75**
Inorganic element :	
Potassium	-0.41
Calcium	0.14
Magnesium	-0.19
Iron	-0.43
Silica	0.22
Extractive contents :	
n-hexane	-0.69**
EtOAc	-0.94**
MeOH	-0.59**
Cold-water	-0.05
Hot water	-0.55*
Component contents :	
Desoxylapachol	-0.06
Lapachol	0.25
Palmitic acid	0.25
Isodesoxylapachol	-0.19
Tectoquinone	-0.62**
Unknown 1	0.55*
Unknown 2	0.03
2-tert-buthyl-anthraquinone	-0.24
Squalene	0.74**
Tectol	-0.03

Note ** = significant at 1 % level * = significant at 5 % level

Fig. 10. Cross-section of black streaked heartwood from a teak tree.



(11a)



(11b)

Fig. 11a-b. The color properties in the the normal and black streak parts. Mean of 6 trees (strong black streaked), 7 trees (thin black streaked), and 5 trees (normal), with the standard deviation in parentheses. The same letters on the same graphic are not statistically different at $p < 0.05$ by Duncan's test.

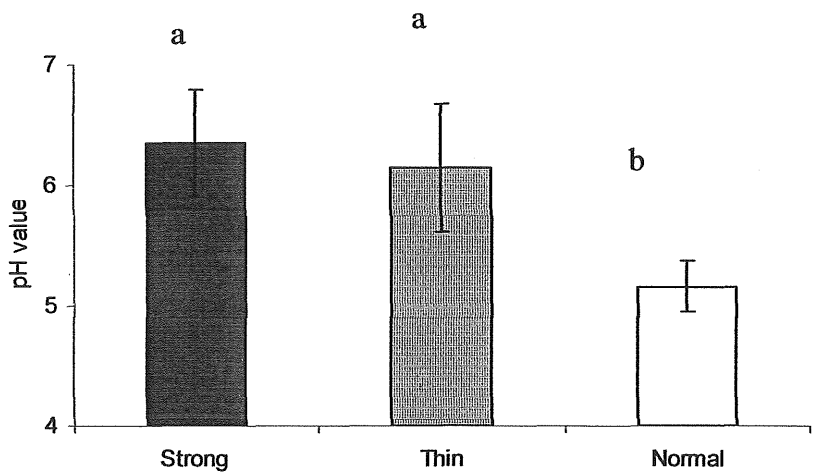
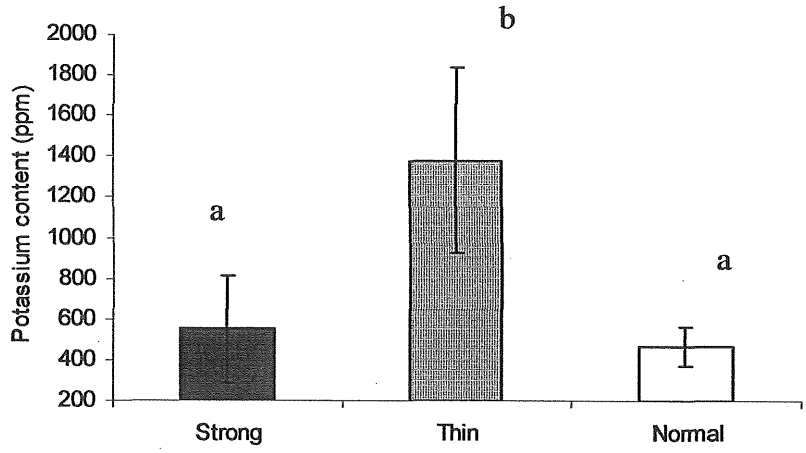
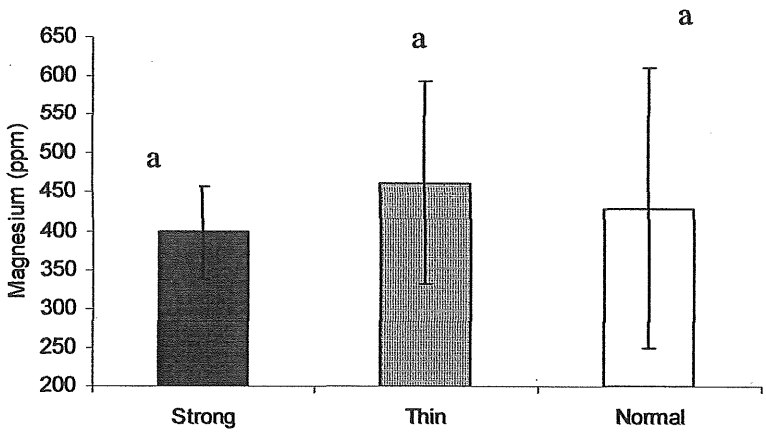


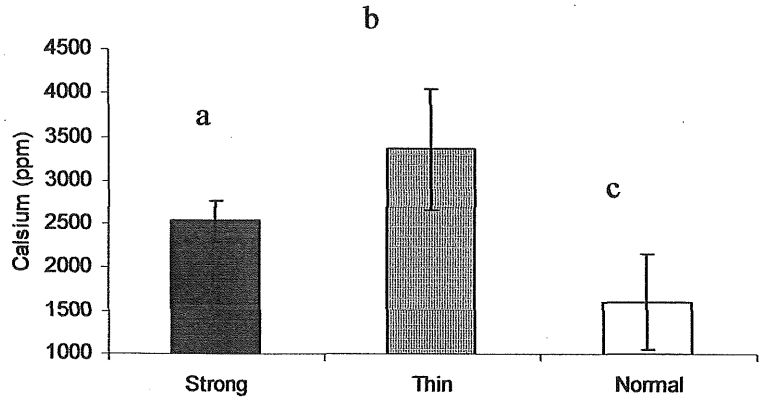
Figure 12. The pH values in the the normal and black streak parts. Mean of 6 trees (strong black streaked), 7 trees (thin black streaked), and 5 trees (normal), with the standard deviation in parentheses. The same letters on the same graphics are not statistically different at $p < 0.05$ by Duncan's test.


Potassium content (ppm)
2000
1800
1600
1400
1200
1000
800
600
400
200
a
b
a
Strong
Thin
Normal

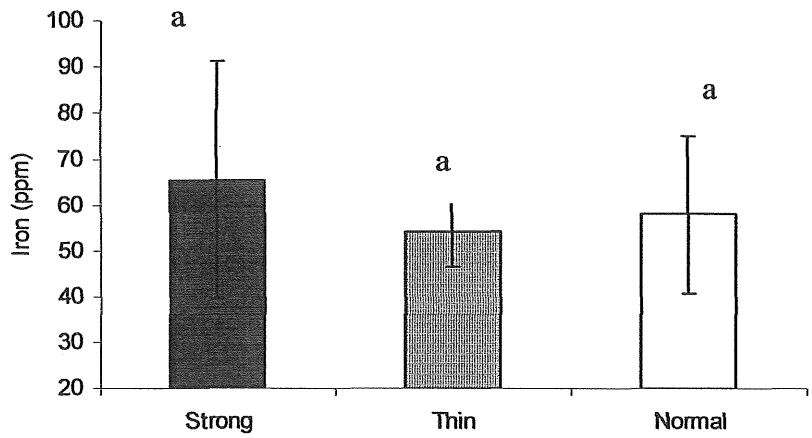
(13a)


Magnesium (ppm)
650
600
550
500
450
400
350
300
250
200
a
a
a
Strong
Thin
Normal

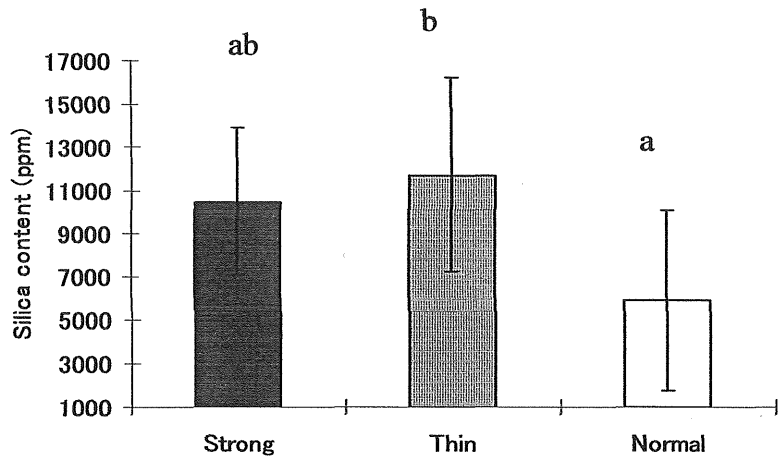
(13b)


Calsium (ppm)
4500
4000
3500
3000
2500
2000
1500
1000
a
b
c
Strong
Thin
Normal

(13c)



(13d)



(13e)

Figure 13 a-e. The inorganic element contents in the the normal and black streak parts (% based on dry wood). Mean of 6 trees (strong black streaked), 7 trees (thin black streaked), and 5 trees (normal), with the standard deviation in parentheses. The same letters on the same graphic are not statistically different at $p < 0.05$ by Duncan's test.

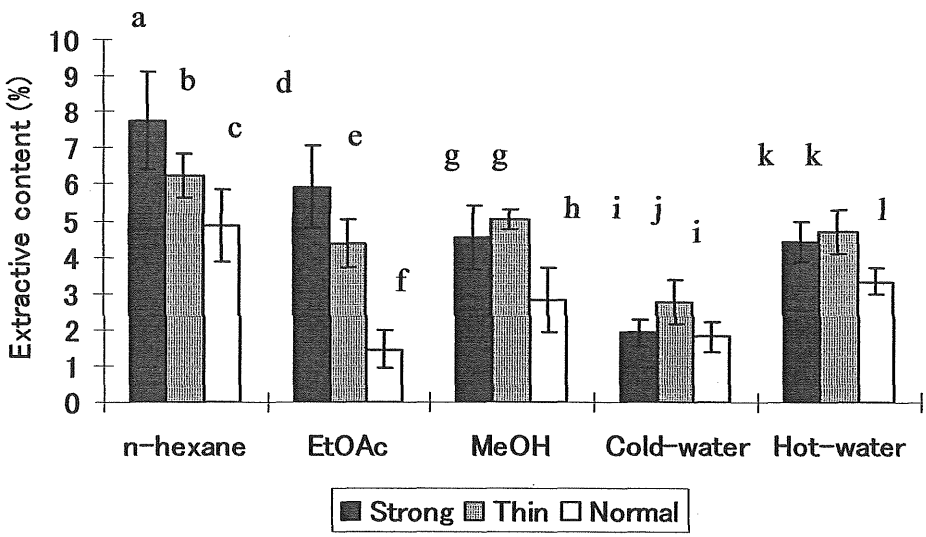
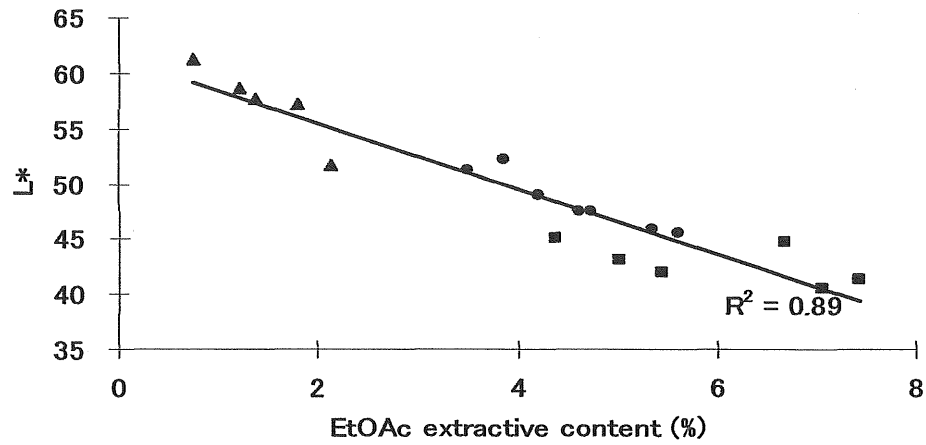


Figure 14. The extractive contents in the the normal and black streak parts (% based on dry wood). Mean of 6 trees (strong black streaked), 7 trees (thin black streaked), and 5 trees (normal), with the standard deviation in parentheses. The same letters are not statistically different at $p < 0.05$ by Duncan's test.



Δ = normal ◊ = thin □ = strong

Figure 15. Scatter diagram between brightness value (L*) and ethyl acetate extractive content.

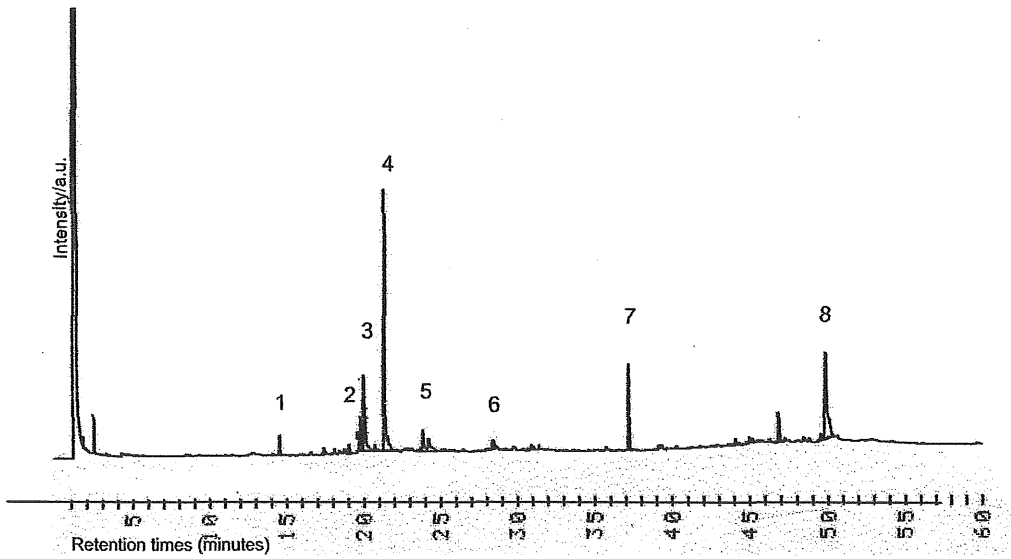


Figure 16. Identification of desoxylapachol or its isomer (peak 1 and 3), lapachol (peak 2), tectoquinone (peak 4), unknown compound 1 (peak 5), 2-hydroxymethyl anthraquinone (peak 6), squalene (peak 7), and tectol (peak 9) by means of GLC of the *n*-hexane extract of black streaked heartwood. Palmitic acid, unknown 2, and unknown 3 were detected at about 19, 21 and 26 minutes, respectively.

Table 8. The amount of major components in the the normal and black streak parts (% based on dry wood). Mean of 6 trees (strong black streaked), 7 trees (thin black streaked), and 5 trees (normal), with the standard deviation in parentheses. The same letters on the same row are not statistically different at $p < 0.05$ by Duncan's test.

Components	Strong	Thin	Normal
Desoxylapachol	0.21 (0.11) a	0.87 (0.79) a	0.34 (0.35) a
Palmitic acid	0.06 (0.02) a	0.10 (0.04) a	0.23 (0.26) a
Lapachol	0.05 (0.05)a	0.30 (0.29) a	0.22 (0.18) a
Isodesoxylapachol	1.20 (0.58) ab	1.68 (1.06) a	0.45 (0.21) b
Unknown 1	0.06 (0.04) ab	0.07 (0.03) a	0.02 (0.02) b
Tectoquinone	2.42 (1.26) a	1.87 (0.81) a	0.41 (0.24) b
Unknown 2	nd	nd	0.14 (0.25) a
Unknown 3	0.12 (0.05) a	0.13 (0.10) a	0.11 (0.06) a
2-tert-buthyl-anthraquinone	0.16 (0.31) a	0.10 (0.16) a	0.02 (0.01) a
Squalene	0.55 (0.09) a	0.83 (0.44) a	1.81 (1.04) b
Tectol	0.85 (0.35) a	0.73 (0.37) a	0.79 (0.32) a

Note : nd =not detected

Chapter 5

Discolored compounds from the black streaked heartwood of teak

5.1. INTRODUCTION

Teak is an economically important tree species indigenous to Java. One of the values of teak wood particularly depends on its aesthetic properties. Coloured wood is in great demand for plywood, furniture and handy craft products. Heartwood color of normal teak is dark brown, but abnormal color due to blackness is not desirable by the people. This discoloration is serious problem that decreases the value of teak products.

It is commonly said that the phenolic compounds of wood are closely related to the coloration. Unfortunately, the phenols of teak related to the heartwood color are remained unknown. In order to investigate the cause of discoloration, it is expected to identify the extractives contributing to the discoloration and to understand their conversion mechanism. Previous chapter showed that ethyl acetate (EtOAc) fraction of teak extractives from successively extraction highly correlated with the darkness of heartwood. In this paper, the compounds isolated and the significance of coloration is described.

5.2. MATERIAL AND METHOD

5.2.1. General

5.2.1.1. Chromatography and physical property determinations

Si-gel 20-40 mesh (Wako) and 63-210 mesh spherical neutral (Kanto chemical) were used for flash and column chromatography, respectively. Precoated aluminum sheets silica gel 60 F_{254} (Merck) were used for TLC. Spots were visualized by UV light irradiation (λ_{254} nm and λ_{360} nm) and by spraying with vaniline-sulfuric acid (for color test) followed by heating at 110 ^{0}C for 10 minutes. Developing solvents used for TLC were

hexane/acetone (1:5, v/v). Melting points were determined on a YANACO Micro Melting Point Apparatus.

5.2.1.2. Spectrum determinations

The ^{13}C (in in 400 MHz), ^{1}H NMR (in 100 MHz) and COSY spectra were determined by a JEOL JNX-400 spectrometer. Chemical shifts are given as δ (ppm) values with TMS as internal standard. Coupling constants (*J*) are given in [Hz]. GLC and GC-MS analysis were described in the chapter 3. The UV-VIS spectral data were determined with SHIMADZU UV-1600 PC in acetone solution. The amount of 0.1 mg of tested sample was dissolved in 20 ml acetone (reagent grade).

5.2.2. Extraction and isolation

Black streaked wood samples were collected from Randublatung, Central Java Province. Samples were ground in a blender and ground samples, 200 g were extracted with *n*-hexane, EtOAc, and methanol, successively while heated for 6 hour each. The extracts were evaporated to yield 7.62 g (38.1 %), 6.70 g (33.5 %) and 5.63 g (28.1%), respectively. The EtOAc extract (4.28 g) were separated into low molecular weight and and polymeric fractions by column chromatography. The extract was chromatographed on a silica gel column using *n*-hexane and acetone as eluents of increasing polarity. The scheme of separation is displayed in Fig. 17. Fractions 2 to 5 contain an unknown compound (C-1) and tectoquinone, respectively. From fraction 8 to 10, tectol was isolated, whereas from fraction 11 and 12, another unknown compound (C-2) was isolated.

5.2.2.1. Unknown 1 (C-1)

C-1 was isolated as a reddish crystal from repeated column chromatography. Yield : 23 mg; Rf value (solvent n-hexane-acetone = 5 : 1) : 0.40; mp: 98-100 ^{0}C. GC-MS m/z (rel. int.) 240 (16) (M^{+}), 225 (100), 211 (5), and 197 (34). UV-Vis spectrum $\lambda_{max}^{acetone}$: 416 nm. NMR (chloroform-d_1) : δ ^{13}C 19.6, 74.6, 77.2, 80.9, 122.4, 126.1, 126.2, 129.9, 131.7, 133.1, 137.5, 140.9, 149.4.^{1}H 1.22 (*s*, 2H), 1.53 (*s*, 3H), 2.15 (*s*, 3H), 3.44(*s*, 4H), 5.70 (*d*, *J* = 10.1, 1H), 6.63 (*d*, *J* = 9.9, 1H), 7.68 (*m*, 1H), 8.06 (*m*, 1H).

5.2.2.2. Tectol

Tectol (Fig. 19) was crystallized as a white powder from an *n*-hexane and acetone solution. Yield : 176 mg; Rf value (solvent n-hexane-acetone = 5 : 1) : 0.32; color reaction (vanillin-sulfuruic acid reagent) : blue; mp: 214-216 ^{0}C. GC-MS m/z (rel. int.) 450 (98) (M^{+}), 435 (100), 211 (77), and 210 (54). UV-Vis spectrum $\lambda_{max}^{acetone}$: 364 nm. NMR (acetone - d_1): δ ^{13}C 27.4 (C-15, C-15'), 27.6 (C-14, C-14'), 76.3 (C-13, C-13'), 111.6 (C-3, C-3'), 116.8 (C-2, C-2'), 121.7(C-11, C-11'), 122.3(C-8, C-8'), 123.5(C-5, C-5'), 126.0(C-10, C-10'), 126.2(C-7, C-7'), 126.6(C-10, C-10'), 126.8(C-9, C-9'), 130.9(C-12, C-12'), 142.3(C-1, C-1'), 145.7(C-4, C-4'). ^{1}H 1.47(*s*, H-15, H-15'), 1.52(*s*, H-14, H-14'), 5.61 (*d*, *J* = 9.8, H-12, H-12'), 5.90 (*d*, *J* = 9.9, H-11, H-11'), 7.51(*m*, H-7, H-7', H-6, H-6'), 7.71 (*s*, OH), 8.15 (*d*, *J* = 8.1, H-8, H-8'), 8.21(*d*, *J* = 8.4, H-5, H-5').

5.2.2.3. Unknown 2 (C-2)

C-2 was isolated as dark powder from repeated column chromatography. Yield : 33 mg; Rf value (solvent n-hexane-acetone = 5 : 1) : 0.22; mp: 85-87 ^{0}C. GC-MS m/z (rel. int.) 210 (100) (M^{+}), 198 (10), and 182 (55). NMR (chloroform-d_1) δ ^{13}C 19.64, 74.63, 77.23, 80.98, 122.39, 126.14, 126.26, 129.99, 131.77, 133.14, 137.57, 140.99, 149.43, 184.46. ^{1}H 1.23 (*s*, 1H), 1.69 (*s*, 3H), 3.81(*d*, *J* = 6.3, 1H), 3.97 (*t*, 1H), 6.12 (*s*, 2H), 6.44 (*d*, *J* = 5.5, 2H), 7.52 (*m*, 1H), 7.63 (*m*, 1H), 7.74 (*d*, *J* =7.7, 1H), 8.07 (*d*, *J* =7.8, 1H).

5.2.3. Discoloration test

The three samples prepared as mentioned above were dissolved in acetone. The solution was then applied to a TLC plate and left in the laboratory for 5 days. The color of TLC before and after exposure was measured with a CIEL*a*b* system, described in using a colorimeter (NF777). L*a*b* system gives the brightness (L*), redness (a*), and yellowness (b*). The resulting total color difference (ΔE*ab) was evaluated using the following equation : $(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$. Tectoquinone (2-methyl anthraquinone, 25753-31 Kanto Chemical), along with several commercially available compounds, which previously reported to occur in the teak extract (Sandermann and Simatupang 1966),

namely lapachol (142905 Sigma-Aldrich), 2-hydroxymethyl anthraquinone (17241-59-7 Acros Organics) were also measured.

5.2.4. Alkaline and acidic treatment of quinones

The 0.5 ml solution of each compound (1 mg/ml) was placed in a glass tube. 100 microliters of potassium hydrogencarbonate ($KHCO_3$) and acetic acid (CH_3COOH) solution at different concentrations (0.01, 0.1, and 1 %), correspond to pH of 3 – 9, was added to each sample. The color change of each solution was observed after 4 h.

5.3. RESULTS AND DISCUSSION

5.3.1. Identification of components

Teak heartwood meals were extracted with EOAc and treated successively with *n*-hexane and acetone. Polymeric parts were fairly large in the EtOAc extract (52 %). In the low molecular weight parts, a combination of fractional crystallization and chromatographic methods led to the separation of tectol and 2 other unknown compounds. Tectol was also isolated in considerable amount. The identification of tectol was confirmed by comparing the spectra data reported by Lemos et al. (1999). Tectoquinone was detected by GC-MS as one of major compounds.

C-1 was separated as orange crystals and was determined by analyzing its GC-MS molecular ion at *m/z* 240. ^{13}C-NMR spectrum of C-1 showed 13 carbon signals, including three methylene carbons (δ_C 74.6, 77.2, 80.9), three olefinic carbons (δ_C 137.5, 140.9, 149.4), one methyl carbon (δ_C 19.6) and six aromatic carbons (δ_C 122.4, 126.1, 126.2, 129.9, 131.7, 133.1). In the ^{1}H-NMR spectrum, the presences of methylene proton (δ_H 3.44), aromatic proton (δ_H 6.63), methylenedioxy protons (δ_H 5.69, 5.71), and vinyl protons (δ_H 7.68, 8.06) were found.

C-2 was separated as dark powders, and was determined by analyzing its GC-MS molecular ion at *m/z* 210. ^{13}C-NMR spectrum of C-2 showed 13 carbon signals, including carbonyl carbon (δ_C 184.46), two methylene carbons (δ_C 77.23, 80.98), three olefinic

carbons (δ_C 133.14, 137.57, 149.43), two methyl carbons (δ_C 19.64, 74.63) and four aromatic carbons (δ_C 122.39, 126.14, 126.26, 131.77). In the ^{1}H-NMR spectrum, it revealed methylene proton (δ_H 3.81, 3.97), aromatic protons (δ_H 6.12, 6.44), and vinyl protons (δ_H 7.52, 7.63, 7.74, 8.07). Due to the presence of aromatic rings and carbonyl carbons, this compound is suggested to be a naphtaquinone compound. The exact structure of and C-1 and C-2 will be elucidated after finishing the measurements COSY C-H, HMBC, and HMQC- NMR.

5.3.2. Discoloration test

The results of air-oxidation of the compounds are shown in Table 9. It was revealed that tectol changes its color (ΔE^*ab = 27.2) as indicated by the considerable decreasing in brightness and increasing in yellowness index. Lapachol and C-1 moderately changed, whereas tectoquinone slightly changed. The changes of the color in other compounds were small.

5.3.3. Alkaline and acidic treatment of quinones

Compounds were treated in acetic acid and potassium hydrogencarbonate in the pH range of 3 - 9 to investigate the influence of pH values. After the treatment, changes in color and UV-VIS absorption were examined. The results (Table 10, Fig. 18) revealed that C-1, C-2, and lapachol changed their color after alkaline treatment at 0.1 % $KHCO_3$ (pH 8.3), whereas tectol changed its color at 1 % $KHCO_3$ (pH 9.3). Tectol showed no clear absorption spectrum, although tectol changed color to dark green. In acidic condition, only C-1 and lapachol changed their color at acetic acid 0.1 % solution (pH 3.9). C-1 showed absorption maxima at 416 (at pH 3.9 and 8.3) and 520 nm (at pH 9.5). It was concluded that C-1, C-2, and tectol is sensitive to changes in pH, while other quinones hardly change color even at high pH. The pH changes does not correspond to the changes in the color of the heartwood that were caused by acidic or alkaline treatment since the pH of black streak part ranges from 5.5 to 7.0 as described in the previous chapter. Furthermore, the

cause of pH change in the discolored part is remained unknown. Thus, the question about the reason of color changes of woods upon pH change cannot be answered in a definite way.

5.3.4. Relationship between structure and coloration of quinone

Table 9 and 10 shows how quinones are colored with air oxidation, acidic and alkaline treatment. Fig. 19 displayed the structural of the treated compounds. Tectoquinone and other two anthraquinones are remained stable, whereas tectol, C-1, C-2 and lapachol changed their color. It is thought that the color changes are due to the structural differences among those compounds. The structural differences are the structure of double bond conjugated and hydroxyl groups. The structure of tectoquinone, 2-tert-butyl-anthraquinone, 2-hidroxy-methyl-anthraquinone which is not colored, are lack of hydroxyl group and double bond conjugated (Table 11). Thus, it is reasonable to assume that the structural features of a hydroxyl group and a double bond conjugated have a significant effect on quinone coloration. Tectol with two hydroxyl groups and a possibility to extend its double bonds conjugated system, changes its color through air-oxidation and through treatment at pH 9.5. Lapachol with one hydroxyl group and a possibility to extend its double bonds conjugated system changes to weak reddish at pH 3.9 and 8.3 as well as moderately changed in color through air oxidation.

5.3.5. The contribution of components to the discoloration of teak wood

As tectol providing the most intense color on TLC plates and showing the most abrupt decrease in brightness, it could be regarded as a major heartwood color precursor or pigment. Tectol can easily be oxydized, therefore, this could be the first stage of tectol before being polymerized by enzymatic reaction to produce special colored compounds.

The yellowness value of C-1 was decreased considerably after air exposure. This fact is in line with characteristic of black streak part in previous chapter, which has lesser value in yellowness. The sensitivity to pH treatment also suggests that C-1 and C-2 could

be the one of precursors of colored substances although the changes did not occur in the weakly acid range. Structural confirmations of those compounds are underway, and will be reported in the near future. This study proved that tectoquinone is not easily to change its color although this compound was observed in comparatively higher amount. Various constituents other than quinone are found in the teak heartwood, therefore, more detailed investigations are needed.

Table 9. Color changes of compounds after 5-day exposure

Compounds	Color measurement			Color changes after exposure			
	L*	a*	b*	ΔL*	Δa*	Δb*	ΔE*ab
Tectol	91.4	0.6	2.7	-13.1	5.1	23.3	27.2
Tectoquinone	91.7	-1.3	6.4	-0.4	-6.0	4.3	7.3
Lapachol	83.0	9.0	17.6	6.9	-7.9	8.6	13.5
C-1	85.3	4.0	24.7	3.1	-2.5	-10.3	11.0
C-2	84.3	0.1	15.7	1.5	1.3	1.6	2.5
2-tert-butyl-AQ	92.1	0.9	-0.1	-0.3	-0.3	2.1	2.1
2-hidroxy-methyl-AQ	92.1	1.0	-0.1	-0.5	-1.1	3.7	3.8

Note : AQ = anthraquinone

Table 10. Color changes and UV-VIS measurement of compounds after 4-hr acidic and alkaline treatment

Compounds	Treatment					
	Acetic acid			Potassium hydrogen carbonate		
	(pH 2.9)	(pH 3.9)	(pH 5.9)	(pH 6.9)	(pH 8.3)	(pH 9.5)
Tectol	X	X	X	X	X	deep green (362 nm)
Tectoquinone	X	X	X	X	X	X
C-1	X	weak reddish (416 nm)	X	X	weak reddish (416 nm)	reddish (520 nm)
C-2	X	X	X	X	weak brownish	weak brownish
Lapachol	X	weak reddish (378 nm)	X	X	weak reddish (368 nm)	deep reddish (484 nm)
2-hydroxymethyl-AQ	X	X	X	X	X	X
1-tert-butyl-AQ	X	X	X	X	X	X

Note : AQ = anthraquinone X = no color changes

Table 11. Structural differences among the compounds from teak extractives.

Compounds	Structure	
	Hydroxyl group	Extension of double bond conjugated
Tectol	2	2
Tectoquinone	0	0
Lapachol	1	1
2-tert-butyl-AQ	0	0
2-hidroxy-methyl-AQ	1	0

Note : AQ = anthraquinone

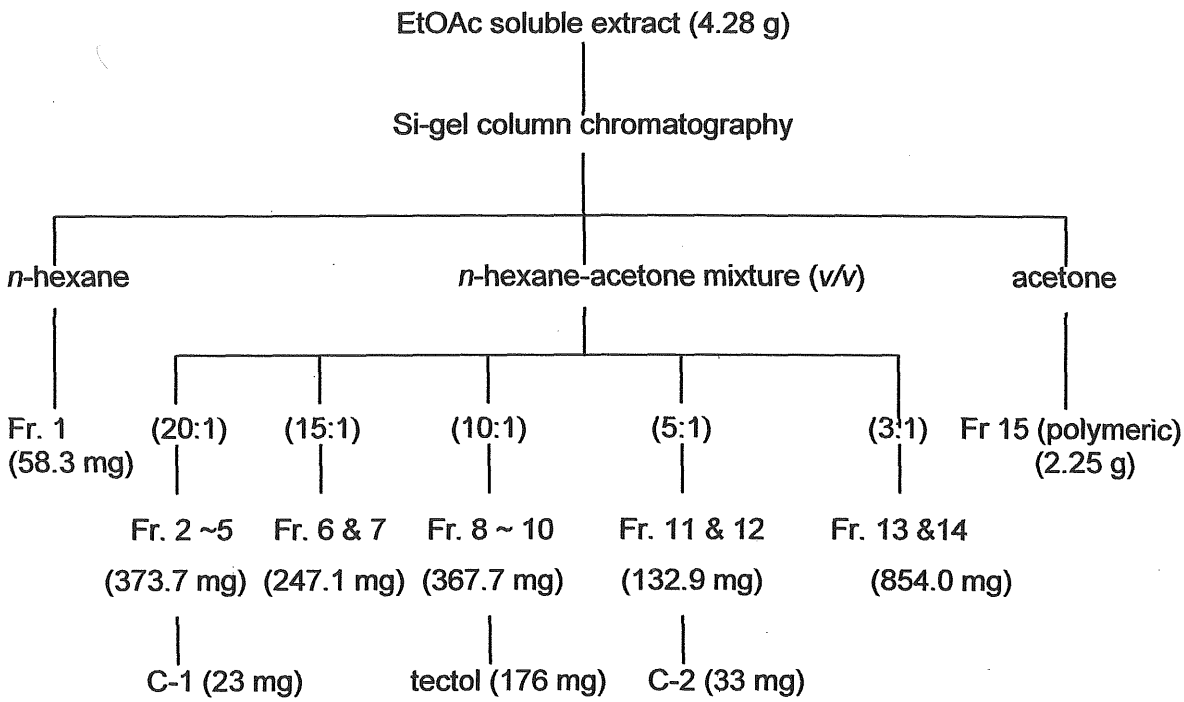


Fig. 17. Scheme of compounds isolation from ethyl acetate soluble extract.

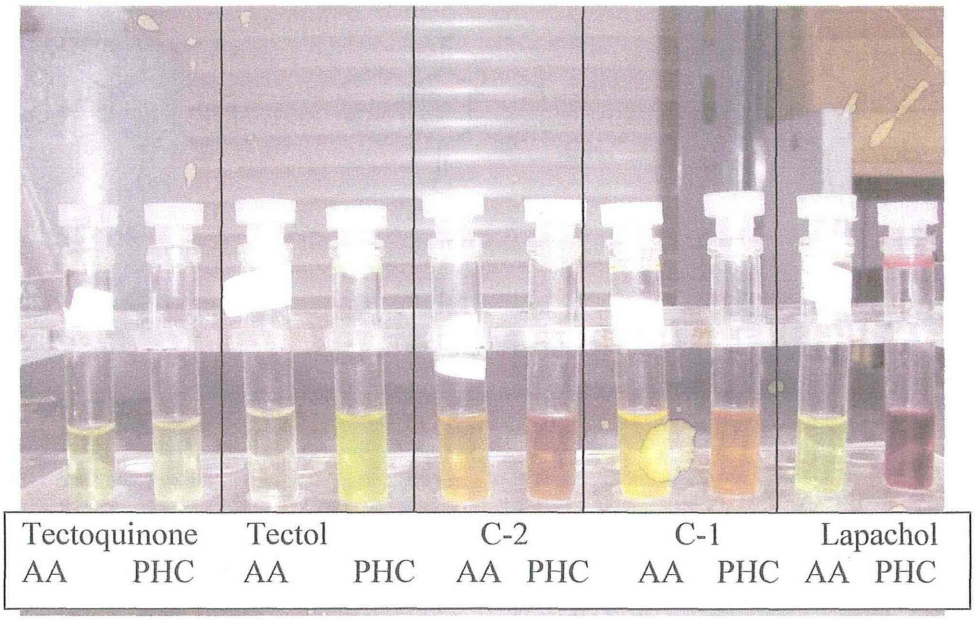
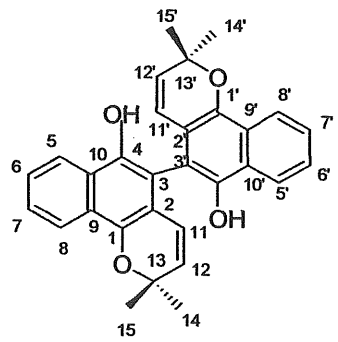
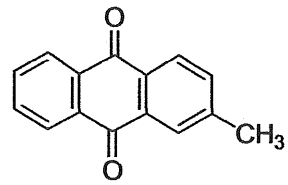


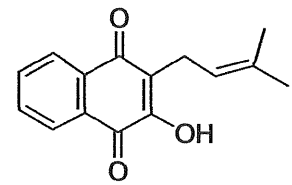
Fig. 18. The color changes of compounds after the treatment of acidic and alkaline. (AA = acetic acid 1 %; PHC = potassium hydrogen carbonate 1 %)



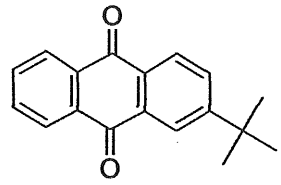
Tectol



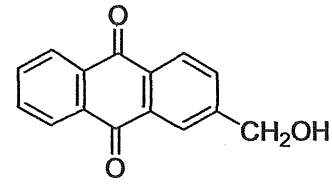
Tectoquinone



Lapachol



2-tert-buthyl-anthraquinone



2-hydroxymethyl-anthraquinone

Fig. 19. Compounds from the extractives of teak heartwood

Chapter 6

General Discussion

6.1. Termite resistance of teak

Much of the economic value of teak comes from is reputation for high termite resistance. This reputation was generated by wood that harvested from old-growth trees. In chapter 2, it has been demonstrated that immature wood (8-year-old trees) was more susceptible against termites attack, both in the heartwood and sapwood. In addition, trees harvested at younger ages will contain a larger percentage of sapwood with lower levels of termite resistance as it was proved in this research. The important result was, in the heartwood of the 30-year-old trees revealed the same termite resistance level compared to that of 51-year-old trees. Therefore, the forest owners should aware of potential importance of rotation age to maintaining high levels of termite resistance.

The survival rate tendency was in agreement with the previous natural termite resistance test (Da Costa et al. 1958; Rudman et al. 1967). It should be noticed that only one termite species was employed in the current study. Perhaps the mass loss after termite exposure was too low to result in significant termite resistance under these test conditions. As it was mentioned, the native termites would be preferred to non-native termites used in this experiment.

To find out the reason for the less resistance, extractives content and color were measured and compared between the age groups. It was observed that there is a tendency which younger teak wood contains less non-polar fraction amount than the older ones. Furthermore, the non-polar soluble fraction (*n*-hexane and EtOAc) and total extractive content of teak wood gave significant correlation with termite resistance characteristics. In other species, extractive contents (cyclohexane-ethanol, ethanol soluble extracts) were related to the decay resistance in *Larix sp* (Windeisen et al. 2002); total phenolic content correlated with decay resistance in *Pinus sylvestris* (Venalainen et. al 2003) and *Larix sp* (Gierlinger et. al 2004b). Moreover, Smith et al. (1989); Anagnost

and Smith (1997); Schultz et al. (1995) found the direct relationship between durability and extractives.

In chapter 3, the distribution of active compounds (tectoquinone, lapachol, desdoxylapachol and its isomer) were measured and correlated with the natural termite properties. Significant differences in desoxylapachol or its isomer content were observed among the outer heartwood of 8, 30 and 51-year old trees, as well as between the inner and outer heartwood parts. Thus, these results suggest that the variability in the total extractive content, total quinone content and individual quinone contents may influence the natural durability level of teak. It was also proved that some active components were detected in the sapwood region, and younger heartwood (8-year-old trees). In addition, the ageing or detoxification by given tree age 51 years was not evidenced in the current study. Unfortunately, the mature trees (older than 80 years) were not characterized for comparison purposes. Therefore, the superiority of trees harvested from long rotation or natural forest to younger trees with respect to the amount of quinone could not be explained here.

As might be expected, the toxic quinone components contents were positively correlated with total extractive content, with the highest correlation degree being observed in isodesoxylapachol content. The amount of tectoquinone and isodesoxylapachol significantly correlated with natural termite resistance properties. Variation in the individual active quinone contents, however, could not thoroughly explain of the variation in natural termite resistance. In this case, the low level of mass loss in the sapwood of 51-year-old trees is unexplained. One possibility is that other substances in the sapwood prevented significant antifeedancy in some samples. That phenomenon reflects the complex nature of teak extractives, as well as, the complex interaction between heartwood extractives and its durability. The other factors, such as the hydrophobicity, synergistic reactions, and wood density are suggested to be involved in some extents.

Considerable variation was observed in the extractive component contents of wood samples taken from the same site. Therefore, this research should be taken as a

preliminary study for plantation teak. Larger number of samples should be provided from different tree ages, different site conditions (plantation - farming land), different climatic conditions (high precipitation - low precipitation), different seed origins, even from different island (Java - outside Java) to describe the nature of plantation teak in Indonesia. Further, the possibility on the chemotaxonomy study of teak wood based on the quinones should be considered. Understandings the causes behind these differences may make it possible to manage for high quinone content, thereby reducing the importance of rotation age to antitermitic activity properties and quinone content.

Significant differences in color parameters between the age groups were evidenced (Chapter 2). However, this study found that not all the wood of younger trees is paler than that of older trees. The correlation between the color properties and extractive content in teak was beyond the scope of this study. Due to the sampling method used in this experiment, it is thought that the color variation partly correlated with the site condition. Therefore, some inconsistencies occur in the results. From this experiment, the moderate correlation between the natural termite resistance parameters was obtained. This is the first study to find out the link between the color characteristics and natural termite resistance properties. The relationship between color, yield and decay resistance has already been studied in other species (Da Costa et al. 1962; Nelson and Heather 1972; Gierlinger et al. 2004a; Amusant et al. 2004).

6.2. Color and chemical characterization of discolored heartwood

This is the first study of characterization black streaked heartwood of teak. Such studies could be very useful for reducing discolorations during tree growing in the future. In chapter 4, it has been proved that the color and chemical characteristics between the black streaked and and wood are significantly differed in extractive and its component contents, pH, and calcium content. The differences between strong and thin black streaked heartwood were also observed. In the chapter 5 showed that tectol provided dark color by air oxidation and by acidic or alkaline treatment.

From previous reports, an increase in inorganic components in discolored tissues was observed (Hart 1968). The discolored woods showed increase in moisture and inorganic elements (Kubo and Ataka 1998) and pH (Takahashi 1996 and Starck et al. 1984). Therefore, it could be concluded the blackening of teakwood followed the general pattern of discolored tissues. Unfortunately, the moisture content of the discolored and normal wood was not measured in this experiment, as well as, the participation of calcium content to the blackening was not attempted. Moreover, the direct effect of the pH was also not obviously proved in this study. It might be that phenolic compounds in the heartwood are oxidatively polymerized under a weakly acidic condition.

The higher extractive contents of discolored heartwood to those of normal wood mean that both high and low molecular weight substances could be studied using HPLC and GC-MS analysis. The significantly high *n*-hexane and EtOAc extractive content in the black streak part indicated that the intense production of pigmented heartwood substances. It was also observed that the final color of the wood after extraction was still darker than that of the normal heartwood. Above a certain degree a polymerization, some compounds remain bound to the matrix. Cross-linking with lignin moiety of wood is also possible. To understand more about the background of the blackening, the acidolysis treatment might be necessary to characterize the polymeric parts, as well as, enzymatic or derivation treatment to the low molecular weight substances.

The blackening process was assumed to be a kind of protective function against biological origins. This hypothesis was supported by the comparatively high level of tectoquinone in that region. However, tectoquinone was relatively stable at low and high pH, as well as in air-oxidation. In studying the blackening *Diospyros kaki,* Yasue et al. (1975) observed that a quinone compound is indicated as a precursor of polymeric substances. On the other hand, tectol, a dimeric naphtaquinone, provided dark colored after air oxidation. Thus, it is proved that the structural of quinone will affect the ability to change its color. Unfortunately, two isolated compounds remain unidentified, as well as,

another dimeric quinone was not isolated, therefore, the conclusions with regard to structural differences of quinone could not be drawn more thoroughly.

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Summary

1. Natural durability of plantation teak

Tree age is one of the most important factors to affect the natural durability of wood. To meet the industrial demand, recently, trees harvested at relatively young ages are increasing. Unfortunately, research data on short-rotation grown teak are very limited. The purpose of this study was to determine the natural termite resistance of heartwood and sapwood of teak (*Tectona grandis* Linn fil.) for trees aged 8, 30 and 51 years. *Reticulitermes speratus* Kolbe was employed as a test termite using a no-choice feeding method during a 14-day observation. The content of the extractives by successive extraction and the color of the wood (CIE L*a*b* system) were also determined.

The recent findings showed that the survival rate and mass loss level due to termite exposition is dependant on the interaction of tree age and radial position. The survival rate and mass loss levels of sapwood and heartwood of 8-year-old trees are significantly higher than those of 30 and 51-year-old trees. The survival rate and mass loss levels in the heartwood regions of 30 and 51-year-old trees are not statistically different. Considerable between tree variations are found in mass loss even in the same stand.

Ethyl acetate removed the least extractives while *n*-hexane or methanol removed the most extractives in a manner depending upon the part of the wood. This significantly increase of *n*-hexane extractive content and total extractive content levels from inner heartwood to outer heartwood of 51- year-old trees, as well as, in the outer heartwood from 8 to 51-year-old groups indicates the increasing content of total extractive content in the heartwood is mainly attributed to an increase of *n*-hexane extractive content level. Also, the color index values (L*, a* and b*) in the heartwood of 8-year old trees are almost similar to those of 51-year-old trees.

The survival rate in the first week is moderately correlated to L*(brightness), and a*(redness) of the wood. The mass loss is moderately correlated to *n*-hexane extractive content, total extractive content, L*(brightness), and a*(redness) of the wood.

Quinones are primarily responsible for the natural durability of teak. For chemical consideration, therefore, the radial distribution of quinones (tectoquinone, lapachol, desoxylapachol and isodesoxylapachol) and other components in the ethanol-benzene (1:2) extract were measured by means of gas chromatography. From the content tectoquinone, lapachol, desoxylapachol and isodesoxylapachol, the total quinone content was calculated. It was revealed tree age and radial position were shown to influence the presence and amount of quinone components detected in teak extracts. Significant differences in desoxylapachol or isodesoxylapachol content were found among the outer heartwood of 8-, 30- and 51-year old trees, as well as between the inner and outer parts of the heartwood. Thus, these results suggest that the variability in the total extractive content, total quinone content and individual quinone contents may influence the natural durability level of teak. However, considerable variation was observed in the extractive component contents of wood samples taken from the same site.

The toxic quinone components contents were positively correlated with total extractive content, with the highest correlation degree being observed in isodesoxylapachol content. The amount of tectoquinone and isodesoxylapachol significantly correlated with natural termite resistance properties. There was no significant relationship between the content lapachol or desoxylapachol and natural termite resistance parameters. In addition, the degree of correlation between the natural termite resistance characteristics and total quinone content was not as strong as might be expected. Variation in the individual active quinone contents, however, could not adequately explain of the variation in natural termite resistance. This fact is assumed to be the complex nature of teak extractives as well as the complex interaction between heartwood extractives and its durability.

2. Characteristics of discolored heartwood

Black streak discolorations in the heartwood of teak may lead to considerable economic loss. Unfortunately, the actual properties of the discolored wood remain unknown as yet. A comparison was made of color and chemical differences between black heartwood (BH) and normal heartwood in teak. The variously BH disks were grouped into two types by visual inspection and L* (brightness) value measurement: strong BH (L* < 45, trees no 1 - 6) and thin BH (L * $\geq$ 45, trees no 7-13). In addition, five disks were obtained from different trees with normal heartwood in the same sites (trees no 14 to 18).

The BH was less than 12-15 units in L* (brightness) value than the normal heartwood. The BH also showed a significant a*(redder value) but lower b*(yellow), hue and chroma values than that found for normal heartwood. The pH and calcium levels of the BH were slightly higher than that of normal wood. Furthermore, this portion gave appreciably higher *n*-hexane and ethyl acetate extractive contents compared to those in normal wood. The differences between normal and BH in methanol and hot water extractive content were significant even though there was no significant difference between the strong and thin BH. Chemical analyses showed that the BH contained significantly higher tectoquinone content but lower in squalene content than that of normal heartwood. The conspicuously high level in tectoquinone suggests the blackening of teak to be connected to a protective function.

The L* (brightness) was moderately correlated to pH value, tectoquinone content, squalene content, *n*-hexane, methanol, and hot-water in Pearson's correlation. Strong degrees of correlation were found between the L* (brightness) and ethyl acetate extractive content as well as between the L* (brightness) and some color characteristics (b*(yellowness), hue and chrome). No significant correlation was measured between the inorganic elements and L* (brightness) value.

In order to investigate the cause of discoloration, it is expected to identify the extractives contributing to the discoloration and to understand their conversion mechanism. As the ethyl acetate extract content was strongly correlated to the L* (brightness) value, its extract was chromatographed on a silica gel column using *n*-hexane and acetone as eluents of increasing polarity. Tectol, and two unidentified compounds (C-1 and C-2) were isolated, while tectoquinone was detected. To test their significance to discoloration, those compounds and several standard compounds were exposed in the air and treated with different pH (using acetic acid and potassium hydrogen carbonate). Tectol showed considerable decreasing in L* (brightness) and increasing in b*(yellowness) index on a TLC plate after 5-day exposure. C-1, C-2, and lapachol changed their color after alkaline treatment at pH 8.3, whereas tectol changed its color at pH 9.3. In acidic condition, only C-1 and lapachol changed their color at pH 3.9. Tectoquinone was relatively stable at low and high pH, as well as in air-oxidation. It is obvious that the discoloration, generating from air-oxidation of tectol, could be one of the reasons to cause the blackening of the heartwood. On the other hand, these results still could not explain adequately the blackening process since the pH of BH part ranges from 5.5 to 7.0.

Based on the chemical structures, it is hypothesized that the structural features of a hydroxyl group and a double bond conjugated have a significant effect on quinone coloration. It is also suggested that each compound could be involved in wood color changes, either as a pigment or as a precursor.

要　約

１．　チーク植林木の耐久性

樹齢は、木材の耐久性に影響する最も重要な要因の一つである。近年、工業的な需要を満たすために比較的若齢の林木の伐採が増加している。残念なことに、短期造林のチークについての研究が大変不足しているのが現状である。本研究は、異なる樹齢（8, 30, 51 年）におけるチーク（*T. grandis*）心材および辺材の抗蟻性(ヤマトシロアリ（*Reticulitermes speratus*）を試験動物に用いた 14 日間の強制摂食試験)、逐次抽出物の成分構成、材色（CIE L*a*b* system）について明らかにすることを目的とした。

今回の研究でシロアリの生存率と摂食量は、樹齢や半径方向の部位と相互に依存していることが明らかとなった。つまり、8 年生の心材と辺材は，30、51 年生のそれらと比べ生存率，摂食量ともに著しく増加した。ただし、30 年生と 51 年生の心材における生存率と摂食量は、統計的な違いは確認されなかった。なお、同一地域においてさえ、個体間にはかなりの摂食量の違いが認められた。

抽出物については、心材および辺材ともに酢酸エチル抽出物が少なくヘキサンとメタノール抽出物が多かった。51 年生の心材の内側から外側にかけてや 8 年生から 51 年生の外側の心材のヘキサン抽出物と全抽出物に見られる増加は、ヘキサン抽出物の増加が強く寄与していることが示された。なお、心材色（color index values）については、8 年生は 51 年生と比較してもほぼ同程度であった。

抗蟻活性と各要因の相関関係については，生存率(第一週目)は材の L^*（明度）と a^*（赤身）に相関を示し、摂食量はヘキサン抽出物量、全抽出物量、L^*（明度）および a^*（赤身）に相関を示した。

キノン類は、主にチークの耐久性に寄与している。EtOH-benzene（1:2）抽出物の GLC 分析から、チーク材の数種のキノン類（tectoquinone, lapachol, desoxylapachol と isodesoxylapachol）とその他の化合物の半径方向の分布を明らかにした。また、tectoquinone、lapachol、desoxylapachol と isodesoxylapachol の含有量から、全キノン含有量を算出した。これより、樹齢と材部の相違（内心材、外心材、辺材）が、チーク抽出物中のキノン類の存在様式に影響を与えていることが示された。desoxylapachol と isodesoxylapachol の含有量は、8、30、51 年生間(外心材)で有意な違いが確認された。この傾向は、内心材でも同様に確認された。このような結果より、全抽出物量、全キノン含有量あるいは個々のキノン含有量などのチーク材の多様性が、耐久性に影響を及ぼしていることが示唆された。なお、同一地域の試料でも抽出物量に大きな違いが認められた。

有毒性を持つキノン類の含有量は全抽出物量と明確な相関を示し、そして isodesoxylapachol には最も高い相関値が認められた。tectoquinone と isodesoxylapachol は、抗蟻活性との著しい相関を示した。lapachol と desoxylapachol は、抗蟻活性との顕著な相関は確認されなかった。さらに、抗蟻活性と全キノン含有量には、期待していた程の強い相関値が認められなかった。活性を示す個々のキノン類の相違によっても、抗蟻活性の相違を十分に説明することはできなかった。この事実は、心材抽出物と耐久性の複雑な相互作用に見られるように、チーク抽出物性状の複雑のためと推察された。

２．　変色心材の特性

チーク心材の黒色縞状の変色部は重大な経済的損失をもたらしている。残念なことに変色材の実際の特性は依然としてまだ知られていない。そこで、黒色縞状の心材(黒色心材:B.H.)と正常心材における材色と化学成分の違いを比較した。黒色心材試料は、目視およびL^*（明度）から濃色材（L^*＜45, tree No. 1-6），淡色材（L^*≧45, tree No. 7-13）｝の２グループに分けた。また同一地域の正常心材（tree No. 14-18）も入手した。黒色心材は、正常心材と比較して12から15低い明度を示した。黒色心材は顕著なa^*（赤身）で著しく高い値を示したが、b^*（黄身）、色相、彩度は正常心材と比較して低い値を示した。黒色心材のpHとカルシウム量は、正常心材に比べわずかに高い値を示した。さらに、この材のヘキサン抽出物量と酢酸エチル抽出物量は、正常心材に比べてとても高い値であった。正常心材と黒色心材におけるメタノール抽出物量と熱水抽出物量の違いは顕著であったが、濃色材と淡色材の顕著な違いは見られなかった。化学成分においては、正常心材に比べ黒色心材でtectoquinone含有量は顕著に多いが、squalene含有量は低い値を示した。著しく高いtectoquinone量は防御作用と関連したチークの黒色化を示唆した。pH、tectoquinone含有量、squalene含有量、ヘキサン、酢酸エチルおよびメタノール抽出物量と明度には、Pearson検定法によって有意な相関が見られた。L^*（明度）と酢酸エチル抽出物量、さらにはL^*（明度）といくつかの色調（b^*（黄身）、色相および彩度）にそれぞれ強い相関が確認された。無機元素とL^*（明度）の間には顕著な相関は確認されなかった。

黒色心材における変色の原因を解明するためには、変色に関与する抽出成分とそれらの変色機構を明らかにし、理解することが大切である。酢酸エチル抽出物量と明度に強い相関が見られたため、極性を変えながらヘキサンおよびアセトンによるシリカゲルカラムクロマトグラフィーで酢酸エチル抽出物の分画を行った。その結果、tectolと２種類の未同定の化合物（C-1, C-2）を単離し、tectoquinoneを確認した。これらの化合物とともに数種類の標準物質の変色に対する重要性を確かめるために、空気中での暴露と、異なるpH処理（酢酸、炭酸水素カリウム）をおこなった。TLCプレート上の５日間の暴露によって、tectolは明度の大きな減少と黄身の増加を示した。C-1、C-2およびlapacholはpH 8.3のアルカリ処理で、tectolはpH 9.3で変色した。酸性では、C-1およびlapacholのみがpH 3.9で変色した。Tectoquinoneは、空気酸化、酸性、アルカリ性のいずれにおいても相対的に安定であった。tectolの空気酸化によって引き起こされている変色は、心材の黒色化を引き起こさせる原因の一つであることが明らかにされた。一方、黒色心材のpHの5.5から7.0の上昇では、十分に黒色化の過程を説明することはできなかった。

化学構造からは、水酸基や共役二重結合といった特徴的な構造が、キノンの着色に顕著な影響を持つと推定される。さらに、それぞれの化合物が色素や前駆体として材色の変化に関係し得ることも示唆された。

Acknowledgements

First and foremost, I would like to thank to Allah SWT, the Most Merciful for keeping my spirits during pursuing the degree.

Next, my deepest gratitude to my main advisor Prof. Koetsu Takahashi for trusting my ablities and for his excellent guidance throughout this study.

I acknowledge the Ministry of Education, Culture, Sports, Science and Technology, Japan for supporting the finance during this study.

I would like to thank to Dr. Tatsuya Ashitani and my previous advisor Prof. Koichi Ogiyama for their helpful suggestions, critical comments and supports during this study.

I am indebted to my colleagues in Faculty of Forestry Gadjah Mada, Widyanto Nugroho and Tomy Listyanto for providing research samples.

Many thank are due to my friends, Mufti Wibowo, Andri Setyawan, Winanto Indriyatno, Sukmono Susanto and Trisno Aji for sample collecting in Perhutani plantation.

I want to thank all students (Norihisa Kusumoto, Nobuhiro Sekine, etc.) in the Department of Bioenvironment laboratory in Yamagata University, for their kindly helps during this study.

I am also grateful to advisors from United Graduate School of Agricultural Science (UGAS), Iwate University, Prof. Hisayoshi Kofujita (Iwate University), Prof. Masaru Hashimoto (Hirosaki University), Prof. Tetsuya Murayama and Dr. Satoshi Hattori (Yamagata University) for assistances and academic counseling.

I thank to Prof. Tadao Wagatsuma (Yamagata University) for conducting inorganic element analysis.

Finally, I would like to thank to my family in Malang for their endless supports and patience.

List of publications concerning the dissertation

1. Lukmandaru, G., K. Takahashi. 2008. Variation in the natural termite resistance of teak as a function of tree age. Annals of Forest Science. 65:708 p1-p8
2. Lukmandaru, G., K. Takahashi. 2009. Radial distribution of quinones in plantation teak (*Tectona grandis* L.f.). in press.
3. Lukmandaru, G., T. Ashitani, K. Takahashi. Characterization of partially black streaked heartwood in plantation teak. Wood Industry (in submitting).
4. Lukmandaru, G., T. Ashitani, K. Takahashi. Color and chemical characteristics of black streaked heartwood in teak. Holzforschung (in submitting).