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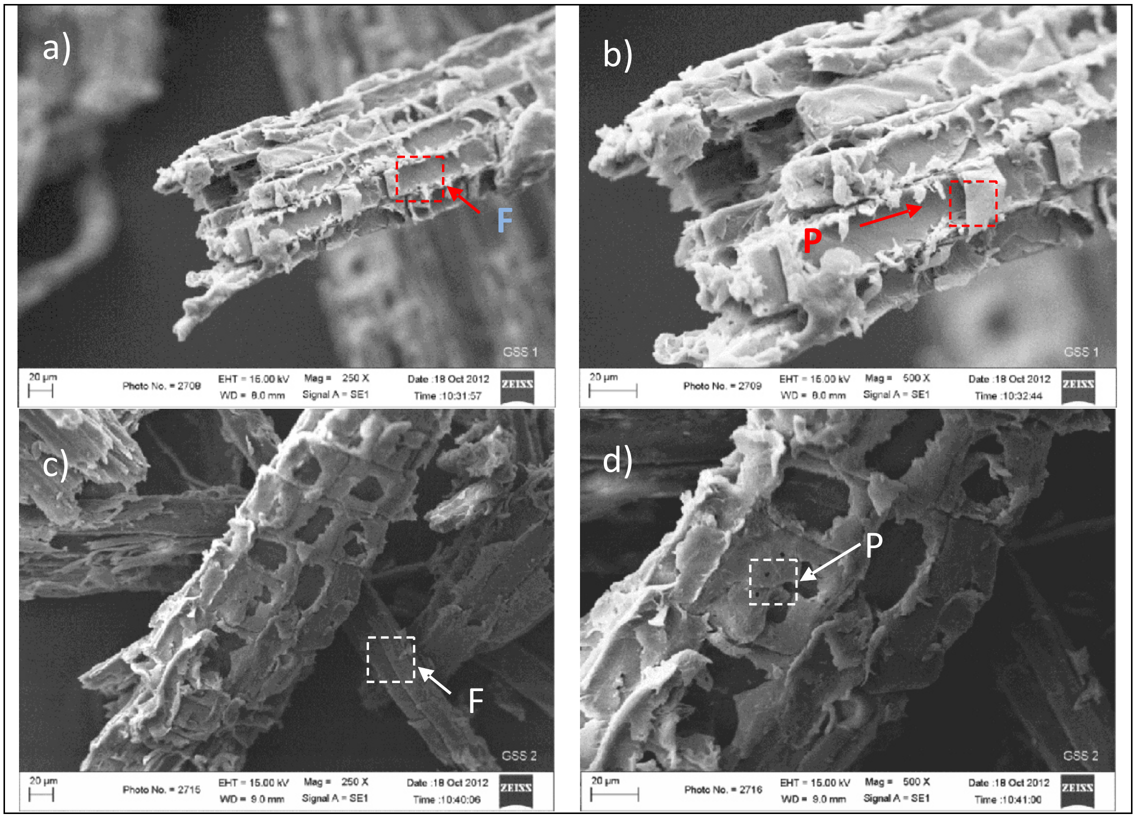

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The Effect of Ultrasonication and Delignification Treatment on the Sugar Released Value of Wood

Ika Wahyuni, Danang Sudarwoko Adi, Lucky Risanto, Fitria, Wahyu Dwianto, Sri Hartati, Rumi Kaida, and Takahisa Hayashi

Abstract

The objective of these research was to study the impact of ultrasonication and delignification pretreatments in several wood species on their easiness for enzymatic hydrolysis, having the potential to be developed as the feedstocks for bioethanol production. Four different wood species from three botanical gardens in Indonesia have been selected, i.e., *Gymnostoma sumatranum*, *Firmiana malayana, Pterocarpus indicus,* and *Alstonia scholaris*, due to their higher sugar released values than a fast-grown tree, Sengon (*Paraserianthes falcataria*), when all were directly enzymatically hydrolyzed without pretreatment. The sugar released values after ultrasonication and enzymatic hydrolysis were between 3 – 5.5 mg/100 mg wood meal. When delignification pretreatment was performed, the sugar released values were higher than those with ultrasonication, ranging between 4 – 10.2 mg/100 mg wood meal. All the sugar released values after pretreatment were higher than those without pretreatment. *Gymnostoma sumatranum* was selected as the most potential wood species in this study due to its consistency among the species producing highest sugar released across different treatments. The SEM results showed that there was no significant changes in the morphological structure of the untreated fiber before and after enzymatic saccharification since it still had a complex structure due to the high lignin content. However, after the delignification treatment, the surface morphology of the fiber showed a decrease in the number of pits of the fiber, the surface residual of pits were reduced, parallel lines were more clearly visible, and the fiber structure was damaged with more small holes presented. The surface morphology of the fiber from the wood powder having delignification pretreatment supports the sugar released values which shows that the these values were higher than other treatments due to more recalcitrant substances were degraded, making it was easier for enzymes to break down cellulose. After ultrasonication, the SEM result showed less disrupted cell wall compared to after delignification which confirmed the higher sugar released data with delignification.

Keywords: bioethanol, delignification, enzymatic saccharification, pretreatment, sugar released, ultrasonication

Introduction

Biofuels, unlike fossil fuels, are potential alternative energy solutions due to their feedstocks' abundant availability, renewability, and sustainability, enhancing energy security. Biofuels have many advantages over fossil fuels especially due to their more environmentally friendly characteristic in reducing greenhouse gas emissions, which is much lower than the emissions released from burning fossil fuels. The use of bioethanol, one form of biofuels, can reduce greenhouse gas emissions by 30~85% when compared to the use of fossil fuels (Fulton *et al*. 2004 in Sainz 2009). Global production and energy use of biofuels increased from 18.2 billion liters in 2000 to 60.6 billion liters in 2007, of which bioethanol is the main supplier of biofuels, accounting for 85% (Coyle 2007 in Sainz 2009). Bioethanol is still widely used as an additive in fossil fuels, but it is possible that bioethanol will replace the function of fossil fuels in the future.

Currently, the raw materials for making bioethanol are still dominated by food crops (first generation bioethanol), including corn, sugar cane, and starch which can threaten world food security. Therefore, alternative sources of raw materials that do not interfere with food security are needed, namely lignocellulosic materials (second generation bioethanol). Lignocellulosic biomass, which can come from forest resources, agricultural and agro-industrial waste, is one of the feedstocks for biofuels and is available in abundance, renewable, and inexpensive. Making bioethanol from lignocellulosic biomass that is financially feasible is a national priority and a scientific challenge.

Many studies on the developments of bioethanol from lignocellulosic materials have been carried out, generally using lignocellulosic waste, such as molasses waste, agricultural residues, and food processing waste. The use of waste can increase its added value and reduce pollution, but on the other hand, there will be a dependency of raw materials for bioethanol industry to other industries, which can disrupt the sustainability of the production process. Lignocellulose from wood can be a solution to this issue. Wood has some advantages over other lignocellulosic materials, such as high cellulose content (up to 80% holocellulose) and it can grow on marginal lands where agricultural crops cannot grow (Kaida *et al*. 2009). Mixing 85% wood-based bioethanol (E85) with fuel can reduce carbon emissions by 65% compared to starch-based bioethanol which only reduces emissions by 17~23% (Watanabe 2008). Bioethanol raw material from wood does not need a storage area as required by other lignocellulosic materials. Timber can also be supplied from industrial forest plantations (*Hutan Tanaman Industri*/HTI) so that the continuous supply of materials can be guaranteed. Cellulose produced from forest plantations reaches 5.0 x 10^9 tons per year which can be converted into 2.6 x 10^{12} L bioethanol (Hayashi 2009). Cultivation of wood on HTI land will be in line with the Kyoto Protocol because the planted trees can increase the amount of carbon stock on earth which in turn will reduce the effect of greenhouse gas emissions in the atmosphere.

Wood cellulose microfibrils consist of a large amount of para-crystal 1,4-β-glucan in the form of nanofibers with a width and thickness of 3 and 4 nm, respectively. The glucan surface of the nanofibers overlaps the hemicellulose, so that each nanofiber is in each glucan layer (O'Sullivan 1997). Nanofibers form a collection of rigid and hydrophobic bundles, resulting in a crystalline region (Hackney *et al*. 1994), while the combination of several para-crystal glucans forms a non-crystalline (amorphous) region.

The conversion of wood into bioethanol is highly dependent on the biomass content. The more biomass content, the greater the possibility of bioethanol produced. Therefore, wood with high biomass content with fast growth is needed, such as fast-grown wood species, while it is also possible that other wood species can also produce high bioethanol yield. Several wood species that have been studied, such as Acacia (*Acacia mangium*) and Sengon (*Paraserianthes falcataria*) have shown different characteristics in producing bioethanol. Acacia has a higher cellulose content than Sengon, but its conversion to bioethanol is lower. The existence of different characteristics of each wood species is an opportunity to find the most suitable wood species to be converted into bioethanol. Since Indonesia has a high diversity of wood species, it can become a major bioethanol producer in the future by exploring many wood species, especially from less commercial and lesser-known species. Research on the possibility of using these various wood species as raw materials for bioethanol is very necessary to provide information about their potential as raw materials for bioethanol production.

The main steps in the process of making bioethanol from lignocellulosic biomass generally start with thermochemical pretreatment, including dilute acid, alkali, ammonia expansion process, and steam explosion process. This aims to free cellulose microfibrils from the lignin matrix and increase their surface area, as well as dissolve hemicellulose, thereby making cellulose more open and easily broken down from polysaccharides into simple sugars in the subsequent enzymatic hydrolysis process (Sanchez and Cardona 2008). Even though many physical and chemical pretreatments to increase the bioconversion of cellulose to glucose have been widely reported, the problems are still revolving around how to improve the conversion of cellulose to glucose units with the help of enzymes, due to the strong crystalline structure of cellulose and its resistance to enzyme attack. The most important thing in fast and thorough enzymatic hydrolysis is the pretreatment of cellulose which can open the cellulose structure and eliminate interactions between glucose chains. Hayashi (2009) stated that wood is very resistant to enzymatic degradation which makes it difficult to degrade into fermentable sugars. Furthermore, the high price of enzymes causes the manufacture of ethanol from wood cellulose to be un-economical.

Delignification and ultrasonication are among many pretreatments that can be used to improve enzymatic saccharification of lignocellulosic biomass. Delignification is required prior to hydrolysis in order to release cellulose and hemicellulose (Sarkar *et al*. 2012). Chlorite delignification for black spruce wood has been reported back in 1970 by Ahlgren and Goring (1971) that showed this treatment could selectively remove lignin during 60% of delignification which afterward would cost the undesired removal of glucomannan and galactan. Lignin is linked to cellulose and hemicellulose in a way that makes the biomass become highly recalcitrant to enzymes and acidified sodium chlorite pretreatment (Wise method) was proven effective in softwood delignification (Kumar *et al*. 2013).

On the other hand, ultrasonication is a possible pretreatment method because it is safe for the environment. Ultrasound creates a hydrodynamic shear force in the aqueous phase that makes it easier for the coarse particles in a slurry to break down into finer particles. This makes more surface area available for enzyme activity (Nitayavardhana *et al.* 2010). Furthermore, the coarser lignocellulosic materials can be broken down into finer particles by using ultrasonication, which significantly increases the surface area for enzymatic attack for bioethanol production. Through the reduction of lignocellulose's structural rigidity and the elimination of mass-transfer resistances, ultrasound can be used to optimize the hydrolysis process and increase product yield while using less time to process and enzyme (Subhedar & Gogate, 2013). When cellulose fibers are subjected to ultrasonication, the fibrils on the surface of the fibers peel off, causing partial fibrillation and subsequent separation of the fibers. In addition, lignin and hemicellulose are released through homolysis of lignin-carbohydrate linkages brought on by sonication (Rehman *et al.* 2013).

Materials and Methods

Materials

There were four wood species used in this study as a result from screening many wood species that have the potential as raw materials for bioethanol, carried out on the collections of woody plants in Cibodas Botanical Gardens (49 species), Purwodadi (32 species), and Eka Karya Bali (20 species). These four wood species were *Gymnostoma sumatranum*, *Firmiana malayana*, *Pterocarpus indicus*, and *Alstonia scholaris*. The raw materials used in this study were collected from the first branches of these wood species. The bark was removed, then crushed using a Hammer mill and Disk mill into wood meal in the size of 40~60 mesh and 115~170 mesh, then dried until it reached air dry conditions.

The chemicals used were ethanol absolute, benzene, sulfuric acid 95~98%, sodium chlorite ($NaClO_2$) 25%, acetic acid glacial 100%, acetone, sodium hydroxide (NaOH) pellet, silica gel, Tween 20, yeast extract (*Saccharomyces cerevisiae*), cellulase, aquades, sodium hydrogen carbonate, ammonium acetate, glucose, di-sodium hydrogen phosphate anhydrous (Na_2HPO_4), sodium arsenate dibasic heptahydrate, copper (II) sulfate pentahydrate, copper (II) chloride dihydrate, potassium sodium tartrate (K-Na Tartrate), citric acid monohydrate, potassium sulfate, ammonium molybdate, calcium choride dehydrate, sodium carbonate anhydrous, and sodium Sulfate.

Equipments

The main equipments used in this research were: (a) Wood sampling equipments: hand saw, circular saw, hammer mill, and disk mill; (b) dimensional measuring equipment (caliper) and weight (digital weigh); (c) laboratory equipments: drying oven, autoclave, centrifugation, incubation, ultrasonication and rotary shaker; and (d) testing equipments: Gas Chromatography (GS) and Scanning Electron Microscopy (SEM).

Methods

Ultrasonic Pretreatment. The wood meal was autoclaved at 120°C for 3 minutes so that it became impregnated with water, washed once with water by centrifugation, then precipitated with 2 ml of water and ultrasonicated for 5 minutes with Branson Sonifer 250. The radiation intensity was adjusted at the microtype limit 7 and duty cycle 50%. The ultrasonicated wood meal would then be used for enzymatic hydrolysis.

Delignification Pretreatment. Delignification of wood meal (100mg) which had been autoclaved at 120°C for 3 minutes was carried out in 3 ml of 8% sodium chlorite solution containing 1.5% acetic acid with stirring at 50 rpm at 35°C for 40 hours, with fresh acid-chlorite solution was added every 8 h. The samples obtained were determined as nondetectable lignin by the Klason method, and then used for enzymatic hydrolysis.

Enzymatic Hydrolysis. A hundred (100) mg of wood meal was autoclaved at 120°C for 3 minutes, so that it was impregnated with water and washed once with water by centrifugation. Commercial cellulase enzymes (Meicelase, Meiji Seika Co., Tokyo, Japan) derived from *Trichoderma viridae* were used to decompose the wood meal. These enzymes contain endocellulases, exocellulases (CBHI and CBHII), xyloglucanase, xylanase, galactanase, and polygalacturonase. Enzymatic hydrolysis of sawdust was carried out in a mixture of 2 ml of 50 mM sodium acetate buffer pH 4.8; 0.02% Tween 20; and 0.4 units of cellulase filter paper (2.0 mg). One filter paper unit was defined as 1 μL glucose per minute released from 50 mg Whatman filter paper at 45°C in a rotary shaker at 135 rpm. Approximately, 100 μL of the supernatant was collected at 24 h after hydrolysis was initiated and used for sugar analysis. The released sugar was measured as reduced sugar using the Nelson-Somogyi method. Furthermore, the released sugar was analyzed directly as alditol acetate using Gas Chromatography (GC).

Scanning Electron Microscope (SEM). The dried xylem was immersed in water under reduced pressure and autoclaved at 120°C for 2 minutes. The wet xylem is then cut by hand using a razor in cross section with a length of about 0.5~1.0 mm. Each part was treated with cellulase preparations used for saccharification, then washed in water three times and dried at 40°C for one night. Each section was observed under a field emission scanning electron microscope (FE-SEM).

Results and Discussion

Chemical Composition of The Wood Species

The chemical components of the four wood species can be seen in Table 1 with their respective botanical gardens' growth location. It can be seen that *P. indicus* has the highest cellulose content, followed by *G. sumatranum*, *F. malayana*, and *A. scholaris*. On the other hand, *G. sumatranum* has the lowest lignin content followed by *P. indicus*, *F. malayana*, and *A. scholaris*.

Tabel 1. Chemical compositions of the wood species

Botanical Garden	Wood species	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Eka Karya Bali	*Alstonia scholaris*	42.9	15.5	26.8
Purwodadi	*Firmiana malayana*	43.2	20.5	25.9
Purwodadi	*Pterocarpus indicus*	47.6	13.1	25.6
Cibodas	*Gymnostoma sumatranum*	43.8	13.5	24.1

Sugar Released after Enzymatic saccharification of Wood Species with and without Pretreatment

Enzymatic Hydrolysis without Pretreatment. Figure 1 shows the comparison of sugar released without pretreatment from several wood species based on the size of wood meal. The highest sugar released was from *P. indicus* for coarse wood meal (40~60 mesh) and *G. sumatranum* for fine wood meal (115~ 170 mesh). The size of the wood meal could affect the conversion of cellulose into sugar released. The finer the wood meal, the higher the conversion. Generally, the enzyme will attack the finer powder first (Zhu *et al*. 2009), while the larger powder will only be hydrolyzed after a few hours of the saccharification process.

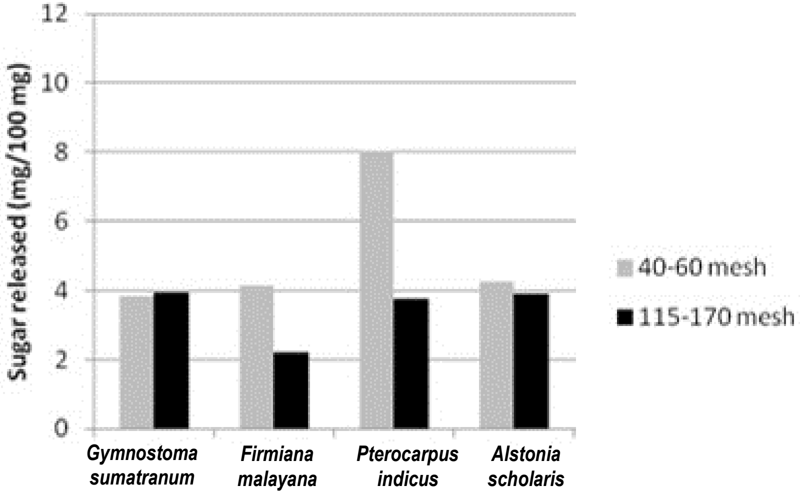

Figure 1. Sugar released without pretreatment.

Enzymatic Hydrolysis after Ultrasonication and Delignification Treatment. The results of sugar released after ultrasonication and delignification pretreatments of the four wood species based on the size of wood meal are shown in Figure 2 and 3. Delignification treatment was carried out to remove lignin from lignocellulosic materials. Lignin is an inhibitor for the action of the cellulase enzyme and is a binder of fibers. From the figures, it can be seen that the powder size has no significant effect on the delignification pretreatment. This was because the impact of lignin removal as an inhibitor of the cellulase enzyme was greater than the powder size during enzymatic hydrolysis. These samples were generally relatively pure cellulose, both in fine and coarse powders, so that the sugar released during the hydrolysis process tended to have not been affected by the particle sizes used in this study.

In Figure 4, it is known that the ultrasonication and delignification pretreatments resulted in higher sugar released than without pretreatment. This was because the cellulose proportions of the pretreated samples were higher than those in the samples without pretreatment due to decreased lignin content. Since lignin can inhibit the enzymatic hydrolysis process, the lower lignin content in the samples after the pretreatments leads to a better process of breaking down cellulose into sugar by enzymes.

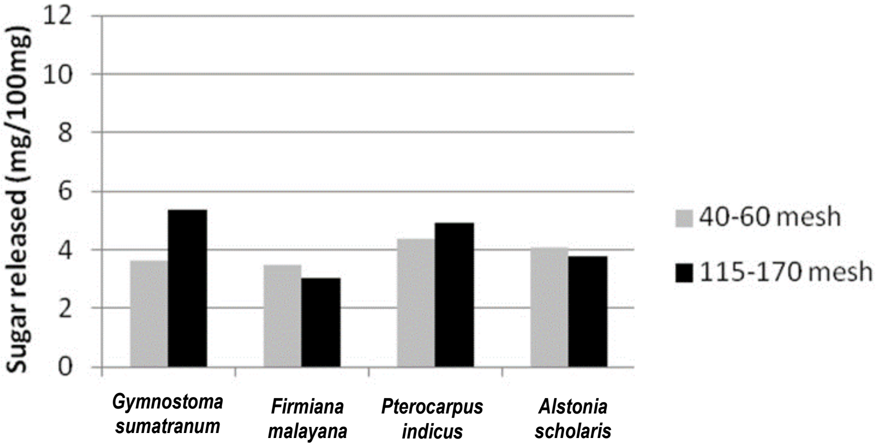

Figure 2. Sugar released after ultrasonication treatment.

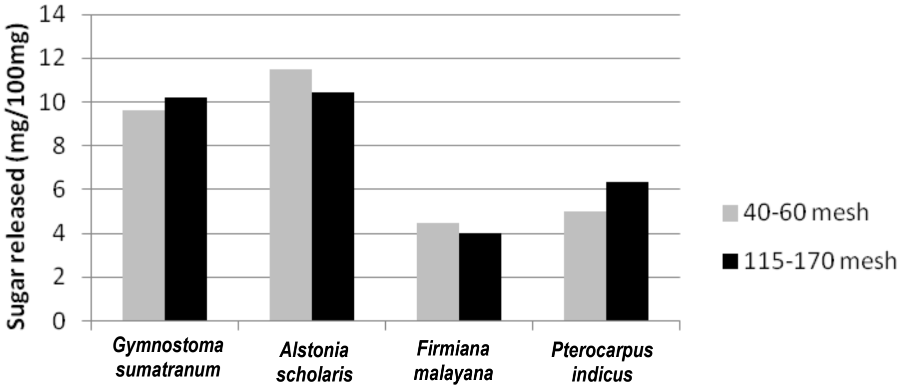

Figure 3. Sugar released after delignification treatment.

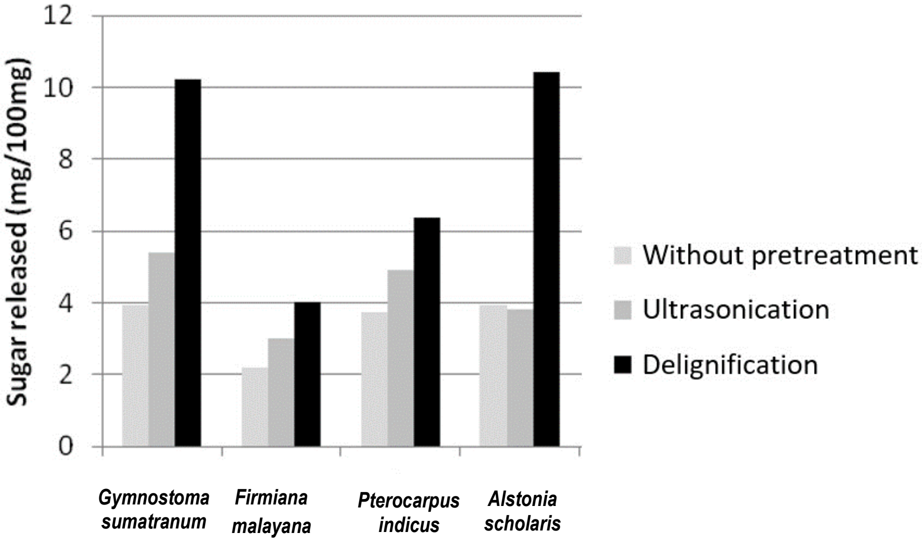

Figure 4. Comparison of sugar released without pretreatment and with ultrasonication and delignification pretreatments (wood meal size 115~170mesh).

Scanning Electron Microscope (SEM)

Based on the results of sugar released with and without pretreatment, *G. sumatranum* wood from Cibodas Botanical Garden was selected as the most potential bioethanol feedstock among the four wood species. Therefore, SEM analyses before and after the ultrasonication and delignification process were performed on this wood to study the morphological changes of the wood fibers and how it affected the subsequent enzymatic saccharification.

Raw Fiber Surface Morphology. In this experiment, two sizes of powder were used, namely 40~60 mesh and 115~170 mesh. The image obtained from the SEM results shows that the fiber surface of the initial test sample consists of 2 main morphologies, namely fiber and pits structure, as shown by arrows F and P in Figure 5. There is no significant morphological difference between the 40~60 mesh and 115~170 mesh size powders. The fiber surface consists of parallel stripes partially covered by residue. Meanwhile, the pits section has a more brittle and fragmented structure, and there are small pores (Figures 5a and 5c).

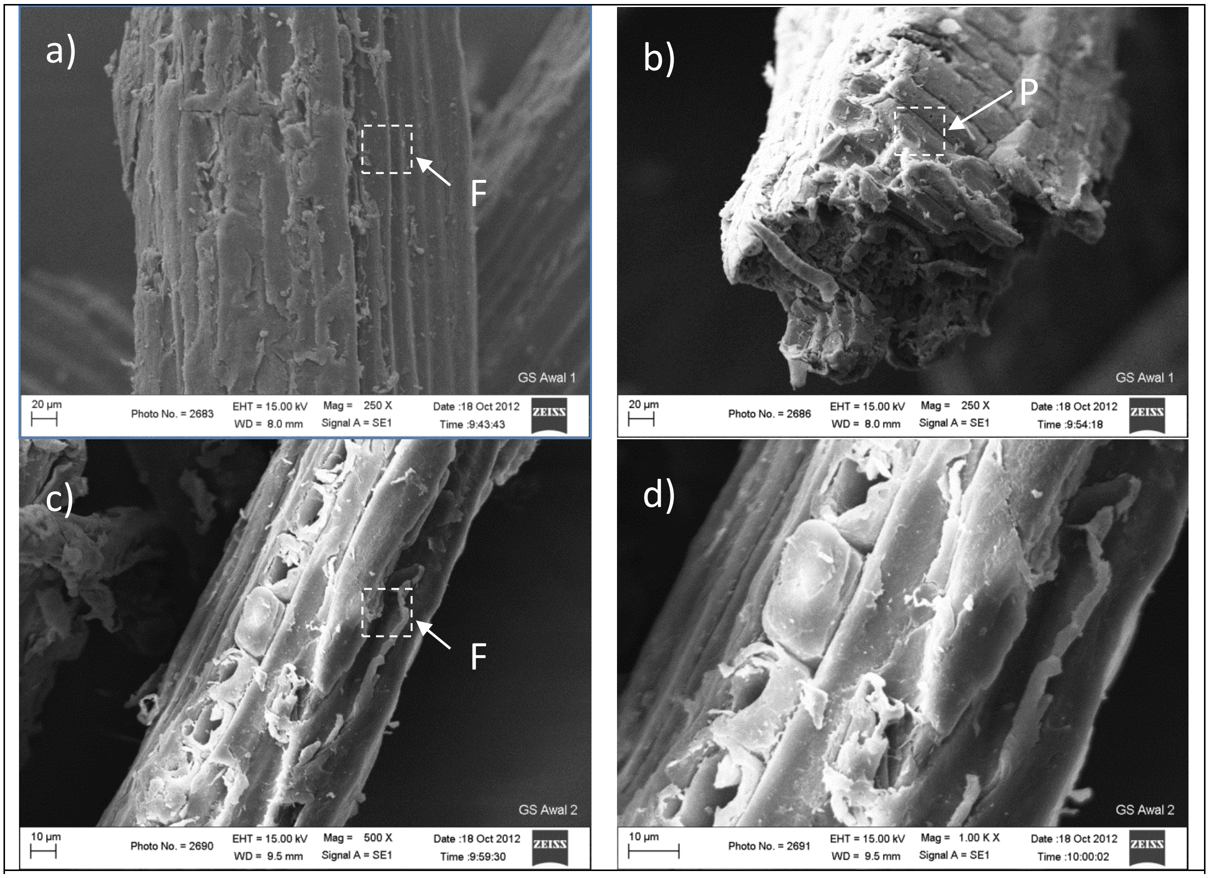

Figure 5. Initial surface morphology of *Gymnostoma sumatranum* (a) Fiber surface section showing parallel lines with residues for 40~60 mesh powder; (b) 40~60 mesh powder pits section; (c) Fiber section showing parallel lines with residues for 115~170 mesh powder; (d) reinforcement of fiber sections.

Fiber Surface Morphology After Enzymatic Hydrolysis without Pretreatment. The SEM results showed that there was no significant changes in the morphological structure of the fibers in the test samples that were not pretreated and just directly hydrolyzed by enzymes (Figure 6) with the raw fibers in Figure 5. This indicates that the untreated wood meal still had a complex structure due to the high levels of lignin, making it is difficult for enzymes to hydrolyze cellulose.

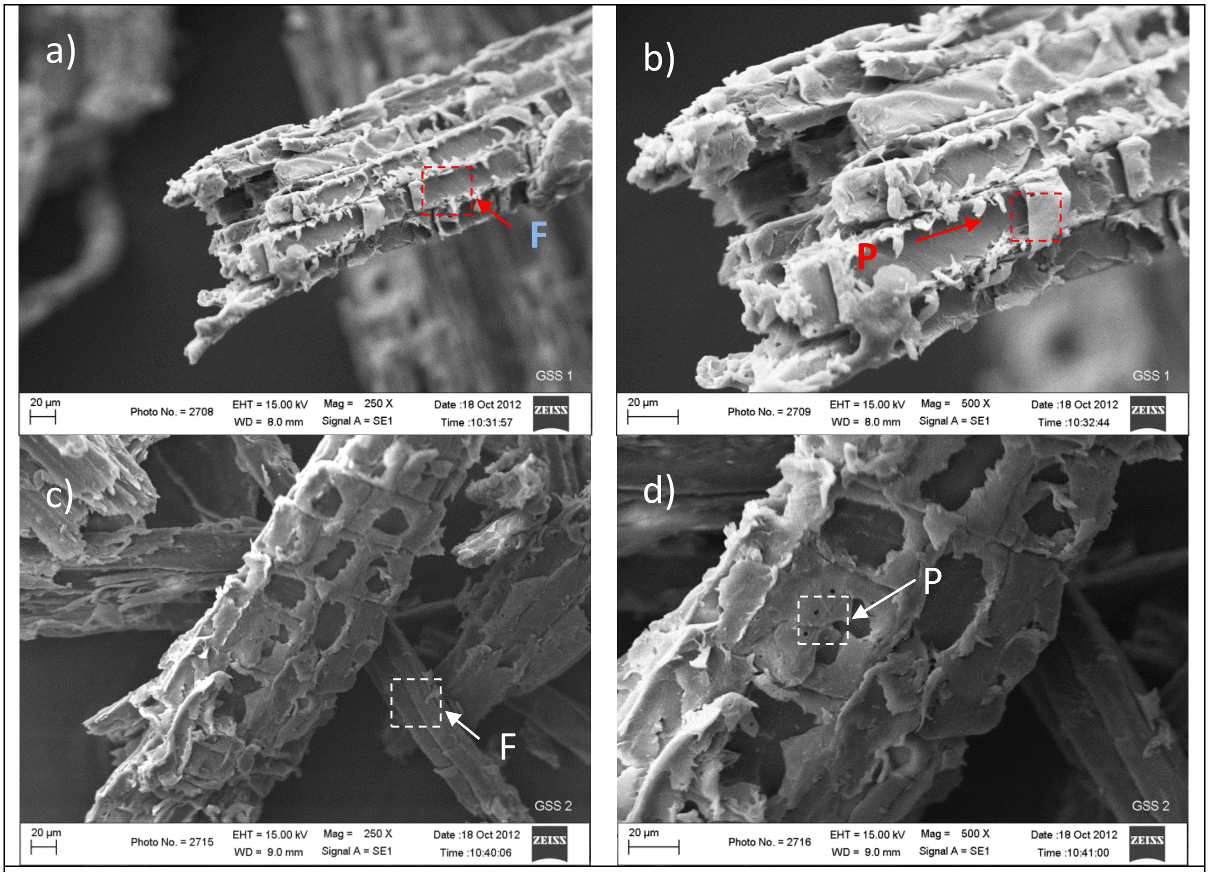

Figure 6. Fiber surface morphology of directly saccharified *Gymnostoma sumatranum* (a) 40~60 mesh powder fiber section; (b) 40~60 mesh powder pits section; (c) 115~170 mesh powder siber section; (d) Pits powder section 115~170 mesh.

Surface Morphology after Ultrasonication and Delignification Pretreatment. Surface morphology after ultrasonication and delignification treatment can be seen in Figures 7 and 8. After ultrasonication (Figure 7), cell ruptures can be clearly seen (Figs. 7a and 7c) and a decrease in pits sections (Figs. 7b and 7c) as reported by other study (Subhedar & Gogate, 2013) that sonication increase surface area due to the cell disintegration. Delignification of sawdust was carried out using 8% $NaClO_2$ solution which has the ability to degrade lignin in wood. After delignification pretreatment, the surface morphology of the fibers showed that there was a decrease in the number of pits sections in the fibers of both powder sizes (Figs. 8a and 8c). This indicates that the pits section is more susceptible to degradation than the fiber structure section. Figures 8b and 8d show that the surface of the fiber section also changes due to the delignification process. Surface residual pitsis reduced and parallel lines are more clearly visible. In addition, the fiber structure was also damaged (small holes) due to some chemical components being degraded by $NaClO_2$. To find out the chemical components that are degraded, it is necessary to carry out further analysis of the chemical components on the powder that has been given delignification treatment. The surface morphology of the fiber from the wood powder that was treated with delignification supported the sugar released data in Figure 4 which shows that the value of sugar released in the powder that was treated with delignification is higher than other treatments, because the more recalcitrant that is degraded, the easier the enzymes break down cellulose.

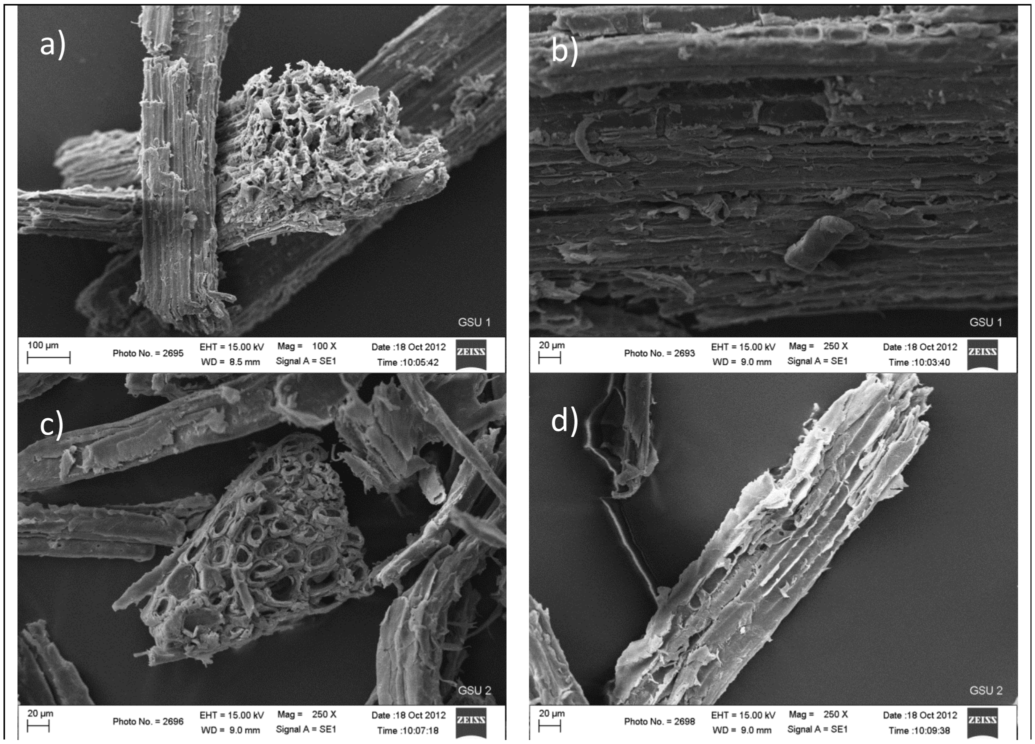

Figure 7. Fiber surface morphology of *Gymnostoma sumatranum* treated with ultrasonication (a) General appearance of 40~60 mesh powder fiber; (b) Reinforcement of 40~60 mesh powder fiber section; (c) General appearance of 115~170 mesh powder; (d) Reinforcement of 115~170 mesh powder fiber section.

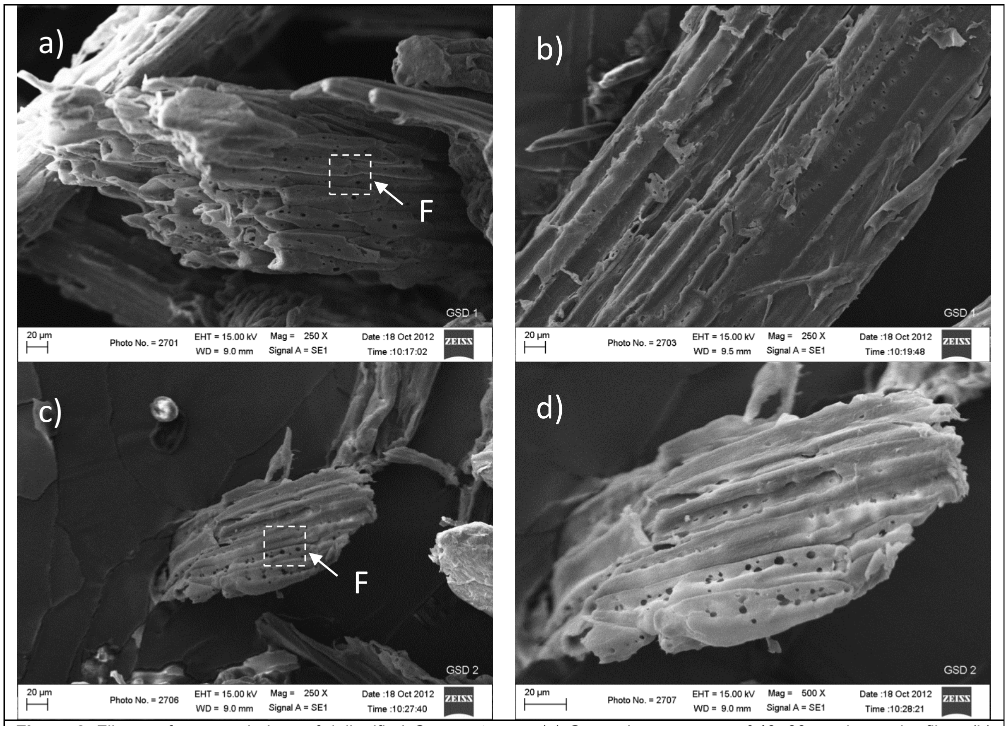

Figure 8. Fiber surface morphology of delignified *Gymnostoma sumatranum* (a) General appearance of 40~60 mesh powder fiber; (b) Reinforcement of 40~60 mesh powder fiber section; (c) General appearance of 115~170 mesh powder fiber; (d) Reinforcement of 115~170 mesh powder fiber section.

Conclusions

Four less commercial and lesser-known wood species from three botanical gardens in Indonesia have been studied for their potential as bioethanol feedstocks. Ultrasonication and delignification pretreatments resulted in higher sugar released after enzymatic hydrolysis than those without pretreatment. Enzymatic saccharification after delignification gave higher sugar released than after ultrasonication, ranging between 3 – 5.5 mg/100 mg wood meal with ultrasonication and between 4 – 10.2 mg/100 mg wood meal after delignification. All the sugar released after pretreatment was higher than those without pretreatment. *G. sumatranum* was selected as the most potential wood species in this study due to its constantly among the species producing highest sugar released among treatments. The SEM results showed that there was no significant change in the morphological structure of the fiber before and after enzymatic hydrolysis as it still had a complex structure due to the high lignin content. However, after the ultrasonication and delignification treatment, the surface morphology of the fiber showed significant changes due to cell disruption, more recalcitrant components were degraded, facilitating easier enzyme penetration to the cellulose. These morphological changes supported the sugar released values where it was higher with pretreatment than without pretreatment.

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Inter-tree Variation in Chemical Components of North Sumatra Benzoin Gum (*Styrax* sp.)

Bagus Praditya Harliando, Sukadaryati, and Ganis Lukmandaru

Abstract

Benzoin gum, which is known as Sumatra benzoin (*Styrax* sp), is widely used as an ingredient in the incense. Although these resins are widely used as flavours and fragrances, no studies have been made on inter-tree variation of its chemical composition. Therefore, benzoin gum samples were tapped from the 30 individual trees (age 10 years, 9~35 cm in diameter) grown in the community forest of Polung, Humbang Hasundutan Regency, North Sumatra. The gum chemical components were analyzed by GC-MS and identified by comparing the fragmentation pattern with the standard components and literature studies. It was found that the major compounds detected by GC-MS were cinnamic acid (51.48%), cinnamyl cinnamate (62.56%), benzoic acid (1.94%), chavicol (5.18%), benzyl cinnamic acid (7.8%), atropic acid (9.84%), and vanillin (1.47%). Two main constituents that were always detected from 30 benzoin trees were chavicol and cinnamic acid, followed by cinnamyl cinnamate (28 trees) and benzoic acid (14 trees). By cluster analysis, 30 samples of benzoin gum can be classified into clusters I - III based on the average chemical components. Cluster I consisted of 7 individual trees with a higher percentage of benzoic acid and chavicol but lower concentration of cinnamic acid compared to other clusters. Cluster II consisted of 18 individual trees with a high percentage of cinnamyl cinnamate whereas clusters III consisted of 5 individual trees characterized with a high percentage of cinnamic acid. By Pearson correlation, it was observed that no significant correlation between the values of diameter and the amount of chemical components of benzoin gum.

Keywords: benzoin gum, chemical components, tree diameter, cinnamyl cinnamate, cluster analysis.

Introduction

Benzoin gum or 'kemenyan' is one of the potential non-timber forest products that have been related to social, cultural and economic values. In Indonesia, benzoin trees grow in natural forests and mostly found in six regencies of North Sumatra (North Tapanuli, South Tapanuli, Humbang Hasundutan, Papak Bharat, Toba Samosir, and Dairi Regencies). There are 4 types of gum benzoin, i.e. toba (*Styrax paralleloneurum*), durame (*Styrax benzoin*), bulu (*Styrax benzoin* var. hiliferum), and siam (*Styrax tonkinensis*). Sumatra benzoin gum that are commercially available are mostly from *S. paralleloneurum,* P. and *S. benzoin,* Dryand. (Kashio and Johnson 2001). Sumatra benzoin is mainly used for incense purpose. *S. benzoin* has been traditionally utilized for the treatment of skin diseases, arthritis, wounds, muscle pain, anxiety, and nervous disorders.

Previous research confirmed that the main chemical compound found in Sumatra benzoin gum is cinnamic acid (Waluyo *et al.* 2006). The cinnamic acid content indicates the level of purity of the benzoin gum. In addition, benzoin gum contains several other compounds such as styrol, vanillin, styracin, coniferyl benzoate, coniferyl cinnamate, benzoeresinol resin, and suma resinotannol (Waluyo and Setiawan 2007). Furthermore, the volatile content of benzoin gum and research on the chemical composition of secondary products of benzoin gum have been discussed in many studies (Fernandez *et al.* 2003; Hovaneissian *et al.* 2006; Modugno *et al.* 2006; and Filippi *et al.* 2009). However, no studies so far have been conducted to explore the inter-tree variation of gum in any Styrax species.

The objective of this study was to determine and to cluster the chemical composition of benzoin gum from 30 trees grown in Humbang Hasundutan Regency, which has long been one of the centers of benzoin gum production. Another objective was to determine the relationship between tree diameter and the percentage of chemical components of benzoin gum.

Materials and Methods

Sample Preparation

Benzoin gum (*Styrax* sp*)* samples were obtained from 30 individual benzoin trees from the Pollung community forest (*ca.* 4 hectares), Humbang Hasundutan Regency, North Sumatra Province. The selected benzoin tree stands (10 years) were at altitude of 1000~1500 m asl. The diameter selected trees ranged from 9-35 cm and previously has never been tapped. The benzoin gum tapping was done at a diameter at breast height using a straight line wound method (vertically) along 3~4 cm from the bark with a depth of wound toward the sapwood of the benzoin tree. The wounds on the benzoin tree were covered and the tree was left for a month to be harvested for the exudate. The harvested benzoin gum was stored in airtight plastic storage. Due to the small amount and wide variation between trees, the gum production in each individual tree was not measured.

Chemical Component Analysis

Benzoin gum (10 mg) was dissolved in benzene (2.5 mg/ml) for 30 minutes. The dissolved benzoin gum was filtered using filter paper and put into the injection vial. Benzoin gum extraction was analyzed for its extractive components using GC-MS (Shimadzu QP-2010) by direct injection. The gas chromatograph temperature conditions used were initial temperature of 80°C, 2 minutes of isotherm at 10°-200°C, with 4 minutes of isotherm at 6°-280°C, with 40 minutes isotherm. The injector temperature was set at 200°C. Helium (He) was used as the carrier gas at a constant flow of 1.2 ml/min. Chemical component identification was based on comparisons between standard components, references (Faust 1992; Bhone *et al.* 2014), and comparison of the sample mass spectrum with spectrum in the NIST Mass Spectral Database in GC-MS analysis. The standard component used was a synthesized cinnamic acid (Faculty of Math and Natural Sciences, Universitas Gadjah Mada). The quantification of each component was calculated by peak relative method.

Statistical Analysis

SPSS version 22 was used for cluster analysis and Pearson analysis. For cluster analysis, selected agglomerative hierarchical clustering (AHC) using the average relationship with the size of the squared Euclidean distance (the root of the standard deviation of each variable) was applied to examine the relationship between populations and their chemical components.

Results and Discussion

Extractive Component Analysis

Figure 1 shows the GC-MS chromatogram from benzoin gum dissolved using benzene. Nine identified components were cinnamic acid, benzoic acid, chavicol, benzyl cinnamate, cinnamyl cinnamate, atropic acid, vanillin, 4-vinylbenzoic acid, and styrene. The two components with the highest peaks were seen at peak 5 (cinnamic acid) and peak 9 (cinnamyl cinnamate). The fragmentation pattern from mass spectrophotometer is presented in Table 1. The comparison of the fragmentation patterns was conducted by comparing the peak ion fragmentation (Table 1).

The peak number 5 (Figure 1) had an identical peak pattern with standard component of cinnamic acid based on the results of the fragmentation pattern. The identification of other components of benzoin gum was conducted by comparing the fragmentation pattern of the benzoin gum with other studies. The mass spectrum of peak number 1 was compared with the spectrum in the earlier report (Bhone *et al.* 2014) and gave the similar results for styrene compounds, whereas peak number 2 gave the same results for benzoic acid compounds after being compared with a study conducted by Faust (1992). By the same method, the mass spectrum of peak number 4 was vanillin (Srivastava *et al.* 2010). The identification of other components of benzoin gum was done by comparing the fragmentation pattern with the NIST and NCBI libraries. The mass spectra of peaks number 3, 6, 7, 8, and 9 were assigned to chavicol, atropic acid, 4-vinylbenzoic acid, benzyl cinnamate, and for cinnamyl cinnamate compounds, respectively.

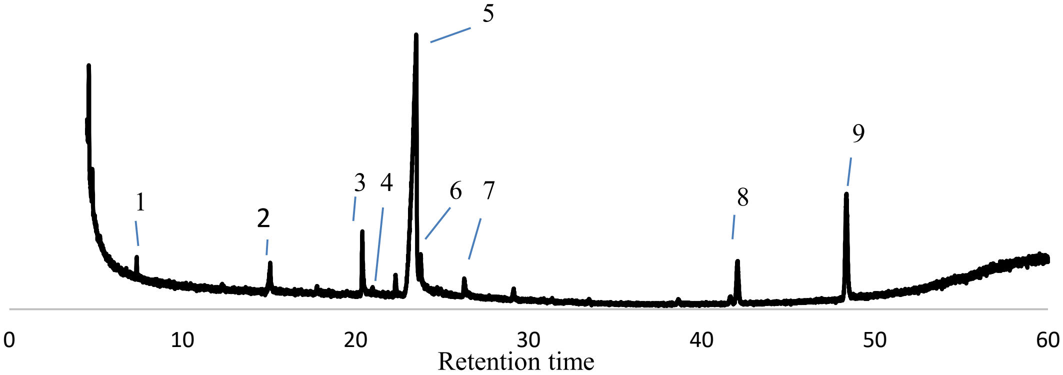

Figure 1. The spectrum of benzoin gum chromatograph; peak 1 (R_t 7.24) = styrene; peak 2 (R_t 15.27) = benzoic acid; peak 3 (R_t 20.77) = chavicol; peak 4 (R_t 22.45) = vanillin; peak 5 (R_t 24.09) = cinnamic acid; peak 6 (R_t 24.10) = atropic acid; peak 7 (R_t 24.65) = 4-vinylbenzoic acid; peak 8 (R_t 43.17) = benzyl cinnamate; peak 9 (R_t 49.41) = cinnamyl cinnamate; and R_t: Retention time (minutes).

Table 1. Peak Ion fragmentation of benzoin gum samples

Peak	Compound	R_T	$[M^+]$	Peak
1	Styrene	7.23	104(100)	**104(100)**. 103(40.2). 92(1). 78(40.3). 63(7). 51(31.8)
2	Benzoic acid	15.27	122(81.8)	122(81.8). **105(100)**. 94(5). 77(48.3) . 65(3.3). 51(19.4)
3	Chavicol	20.80	134(100)	**134(100)**. 115(11.4). 107(26.8). 91(12.10). 77(20.6). 66(8.2). 51(10.2). 39(11)
4	Vanillin	22.45	152(88)	152(88). **151(100)**. 137(12.3). 123(21.8). 109(16.7). 81(38.6). 65(13.3). 53(25.7)
5	Cinnamic acid	24.09	148(71.4)	148(71.4). **147(100)**. 131(19.3). 120(5.1). 103(50.1). 91(19.9). 77(34.3). 63(5.7). 51(26)
6	Atropic acid	24.10	148(51.5)	148(51.5). 131(21.6). 121(17.7). **103(100)**. 91(35.8). 75(52.6). 63(29.9). 51(64.8). 37(12.7)
7	4-Vinylbenzoic acid	24.65	148(100)	**148(100)**. 131(13.6). 118(3.2). 103(38.6). 93(5.8). 78(19.9). 63(17.3). 51(13.7)
8	Benzyl cinnamate	43.17	238(16.4)	238(16.4). 192(62). 178(8.5). 147(7.6). 131(91.9). 115(15.9). 103(41.3). **91(100)**. 77(36.9). 65(16.5). 51(16.2)
9	Cinnamyl cinnamate	49.45	264(3.5)	264(3.5). 219(14.1). **131(100)**. 117(28.7). 115(30.8). 103(23.3). 91(10.8). 77(15.4). 63(1.9). 51(5.9)

Description: R_t = Retention time (minutes); [M+] = Molecular ion; *bold = base peak.

Table 2. The amount of chemical compounds (%) of benzoin gum (*Styrax* sp.)

Compound	Retention Time (minute)	SI (%)	Minimum	Maximum	Mean	Standard Deviation	Tree Diameter ^{a} (cm)	Number of tree ^{b}
Styrene	7.24	92	tr	4.29	0.22	0.82	13.69	3
Benzoic acid	15.27	89	tr	1.94	0.39	0.54	18.79	14
Chavicol	20.77	91	1.61	5.18	3.19	0.98	15.61	30
Vanillin	22.45	90	tr	1.47	0.20	0.46	28.66	5
Cinnamic acid	24.09	97	4.46	51.43	21.68	13.15	10.19	30
Atropic acid	24.10	60	tr	9.84	0.45	1.82	15.61	4
4-Vinylbenzoic acid	24.65	62	tr	5.76	0.58	1.42	13.38	6
Benzyl cinnamate	43.17	91	tr	7.80	0.90	1.82	28.34	9
Cinnamyl cinnamate	49.41	94	tr	62.56	26.42	18.83	20.70	28

Note : SI = Similarity Index (%); n = 30 tree ; a = tree diameters with maximum value of the detected compounds, ^{b} = number of tree the detected compound; *tr= trace (< 0.1 %)

Table 2 presents the summary from the analysis of benzoin gum using GC-MS. Two chemical components of benzoin gum that showed the highest concentration (51.43%) were cinnamic acid (ret. time of 24.09 min.) and cinnamyl cinnamate (62.56%, ret. time of 49.41 min.). Vanillin, styrene, benzoic acid, cinnamic acid, cinnamyl cinnamate, and benzyl cinnamate were previously detected in Sumatra benzoin by HPLC-PAD and GC-MS (Hovaneissian *et al.* 2008) and by HPLC-frit FAB-MS and GC-MS investigations (Pastorova *et al.* 1997). Chavicol, atropic acid, and 4-vinylbenzoic acid were not detected in the earlier reports. The low values of similar index for atropic acid and 4-vinylbenzoic acid should be noticed. Isolation and identification of those peaks in the next works should be conducted to confirm their presences.

No species identification was conducted in this experiment. The number of tree was presented to find out the components distribution regardless the species. Two main components that always detected from 30 benzoin trees are chavicol and cinnamic acid, followed by cinnamyl cinnamate (28 trees) and benzoic acid (14 trees). Chavicol, styrene, and vanillin are from simple neutral aromatic groups, whereas atropic acid, cinnamic acid, benzoic acid, and 4-vinylbenzoic acid are from aromatic carboxylic acids. The esters detected were cinnamyl cinnamate, and benzyl cinnamate.

Cluster and Correlation Analysis

Cluster analysis was applied to all individual data by using a hierarchical method. This method compares the Euclidean distance between individuals as their similarity index. Then, the grouping was performed according to the weighted average linkage method. Cluster analysis was carried out by comparing the 4 main components of benzoin gum with a frequency of more than 10 in each sample of benzoin gum. These components are cinnamic acid, benzoic acid, chavicol and cinnamyl cinnamate. The division of benzoin gum clusters is based on its constituent chemical components. The chemical structures of those components are presented in Figure 2.

Previous report (Waluyo and Setiawan 2007) quantified the cinnamic acid (25~33%) by chemical reaction in Sumatra benzoin from six class qualities. Pastorova et al. (1997) quantified cinnamyl cinnamate (8~14%), methyl cinnamate (10~17%), cinnamic acid (4~7%), and benzyl cinnamate (2~4%) in *S. benzoin* by spectroscopic works. The chemical components detected in more than 10 tree samples were classified into the major components (cinnamic acid, benzoic acid, chavicol, and cinnamyl cinnamate). The chemical components detected in less than 10 tree samples were classified into minor components (4-vinylbenzoic acid, atropic acid, vanillin, benzyl cinnamate and styrene).

The results of the cluster analysis are in the form of dendrograms or tree diagrams which are shown in Figure 3. Cluster I, II, and III consisted of 7, 18, and 5 individual trees, respectively. Table 3 shows the average percentage of the relative content of chemical components in each cluster. Cluster I had a medium cinnamic acid (23.30%) with a low percentage of cinnamyl cinnamate (9.70%). Cluster II had a low cinnamic acid (14.50%) with a high cinnamyl cinnamate (36.43%). Cluster III had a high percentage of cinnamic acid (45.25%) with a medium percentage cinnamyl cinnamate (17.66%). It was found that there were clear differences between cluster I and cluster II or between cluster II and cluster III. The differences can be seen in the percentage of cinnamic acid and cinnamyl cinnamate. Cluster I and cluster III looks similar in chavicol and benzoic acid percentages. By close examination, it turns that cluster I had a higher percentage of benzoic acid and chavicol, but lower cinnamic acid and cinnamic acid compared to other clusters. Individual trees classified in cluster II had a high percentage of cinnamic acid, whereas cluster III had a high percentage of cinnamic acid compared to other clusters.

Numbers in the vertical direction of the dendrogram indicate the diameter of each individual tree in the cluster. The diameter value was evenly distributed in each cluster, indicating that the diameter value has no effect on the percentage and concentration of the chemical components in benzoin gum. This trend was confirmed by correlation analysis (Table 4). It can be seen that the diameter had no significant effect on the percentage of chemical components.

The relationship between tree diameter and yield has been studied for oleoresin in some pine species (Lekha and Sharma 2010) or sap in rubber species (Woelan 2005). It is assumed that trees with bigger diameters are able to produce benzoin gum with high concentrations of chemical components. From the results of the Pearson correlation, it can be seen that there was no correlation between growth rate (diameter) and the amount of chemical components of benzoin gum. It implies that no special treatment is required to increase the growth rate or diameter of the benzoin tree. Unfortunately, the correlation between benzoin tree diameter and quantity of benzoin gum production was not analyzed in this experiment. Therefore, it is not recommended to use individual trees from this study as benzoin gum-producing trees for production purposes.

Table 3. Mean chemical components of cluster I-III of benzoin gum

Cluster	Benzoic acid	Chavicol	Cinnamic acid	Cinnamyl cinnamate
I	1.19 ± 0.4.9	3.95 ± 0.54	23.30 ± 6.32	9.70 ± 10.85
II	0.43 ± 0.19	2.75 ± 0.97	14.50 ± 7.44	36.43 ± 16.52
III	0.96 ± 0.25	3.74 ± 0.51	45.25 ± 5.30	17.66 ± 6.91

Table 4. Pearson analysis of the amount of benzoin gum components and tree diameter

Compound	Pearson Corelation
Styrene	0.01
Benzoic Acid	0.29
Chavicol	-0.09
Vanillin	0.19
Cinnamic acid	0.29
Atropic acid	-0.07
4-Vinylbenzoic acid	0.06
Benzyl cinnamate	0.28
Cinnamyl cinnamate	0.12

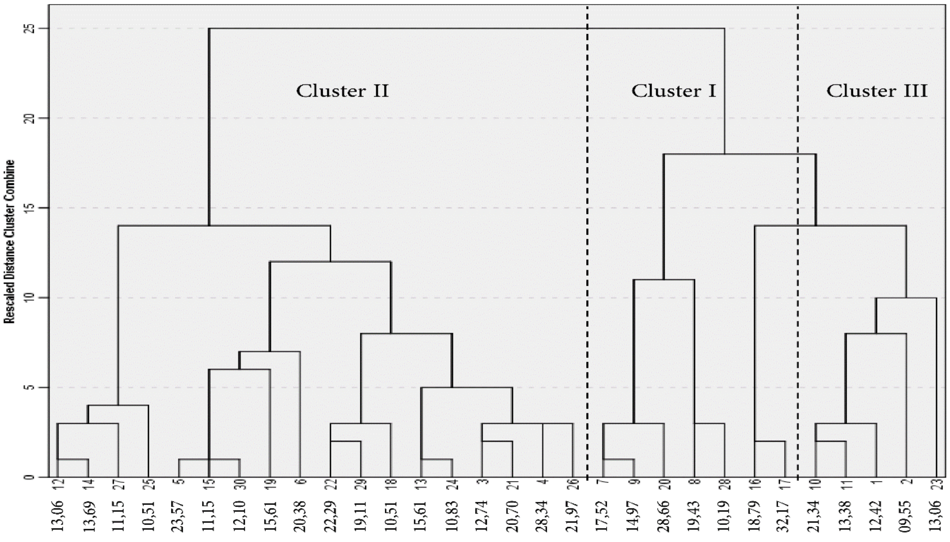

Figure 2. Dendrogram of Benzoin Gum Clusters with X-axis is Tree Diameter (cm)

Conclusion

The results of the GC-MS analysis of benzoin gum on 30 trees (age 10 years) with tree diameters range of 9-35 cm showed cinnamic acid, cinnamyl cinnamate, benzoic acid, chavicol, benzyl cinnamate, atropic acid, vanillin, and 4-vinylbenzoic acid and styrene as the detected components. The major components were cinnamic acid and cinnamyl cinnamate. The results of the cluster analysis of individual trees were grouped into 3 clusters with different percentages of chemical components. Cluster I was classified with average percentage of benzoic acid of 1.19%, chavicol of 3.95%, cinnamic acid of 23.30%, and cinnamyl cinnamic of 6.93%. Cluster II was classified with average percentage of benzoic acid of 0.43%, chavicol (2.75%), cinnamic acid (14.50%) with high cinnamyl cinnamic acid (36.43%). Cluster III was classified with percentage of benzoic acid of 0.96%, chavicol of 3.74%, with high levels of cinnamic acid (45.25%) and cinnamic cinnamic acid (7.66%). There is no significant correlation between the levels of diameter and the amount of chemical components of benzoin gum.

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Chemical Investigation of Methanol Extracts of *Swietenia mahagoni* Leaves and Its Antioxidant Activity

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Abstract

Swietenia mahagoni is among the species of trees used by the Indonesian Government for the purpose of afforestation and timber production through Perhutani Enterprise. The common use of this species as wood products has prompted investigating the chemical properties of its leaves. Based on this background, this study aimed at investigating the methanol extracts of both 2- and 3-year old *S. mahagoni* leaves extractives together with its antioxidants and phenols contents. The antioxidant activity was conducted through the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, while the total phenolic and flavonoid content were measured through Folin-Ciocalteu and $AlCl_3$ methods, respectively. Additionally, 24 compounds were characterized by the GC-MS, and were grouped into phenolics, fatty acids and hydrocarbons, and terpenoids. The total phenolic and flavonoid contents in both 2 and 3 years old leaves of *S. mahagoni* ranged from 36.4 ± 0.84 to 42.0 ± 0.18 mg GAE/g dried extract and from 2.24 ± 0.15 to 18.55 ± 1.05mg QE/g dried extract, respectively. Also, the antioxidant values were 66.45 ± 1.85% and 77.59 ±11.23%, respectively. Based on the results, the antioxidant activity of *S. mahagoni* leaves was indicated as a result of the presence of α-tocopherol and α-tocopherolquinone in the leaves extracts.

Keywords: *S. mahagoni*, *Antioxidant*, *Total phenolic content*, *Total flavonoid content*, *DPPH*

Introduction

Swietenia mahagoni is a tree species introduced to Indonesia from Jamaica (Panda *et al*. 2010; Rahman *et al*. 2014). The presence of this species in this country has enhanced the characterization and identification of some of its properties. The leaves of *S. mahagoni* is a potential source of antioxidant agents, which are the source of phenolic compounds extracted with polar solvent such as methanol (Roy et al. 2009; Al-Radahe et al. 2012; Roy et al. 2014). In order to discover the antioxidants sources from these leaves, there is need to investigate the total phenolic and flavonoid content of its methanol extracts due to the positive correlation between its antioxidant and phenol contents.

This species is used for the purpose of afforestation in Indonesia due to its beneficial roles in terms of ecology, as well as a source of foreign exchange. This species consistently provides evergreen leaves throughout the year, which positively impact timber production, thereby contributing to the economy development of the country. The trees have been extensively used as home building materials, ornamental purposes, and protection in sloppy areas. According Jøker (2000), its wood is excellent for producing quality timber, with density within the range of 560-850 kg/m3 and 15% moisture content, making it a good potential for usage in large scale timber production.

Previous studies on the chemical compositions of the leaves of *S. mahagoni* characterized cyclomahogenol, swiemahogins A, and swiemahogins B as its componenta (Chakraborty *et al.* 1971; Chen *et al.* 2007; Naveen *et al.* 2014). Also, there were reports of some bioactivities from the leaves and seeds such as antifeedant (Mostafa *et al.* 2012), depressant (Rahman *et al.* 2010), antimicrobial (Sharma *et al.* 2011), and antidiabetic (Sathish *et al.* 2010),. Therefore, this study aimed at chemically analysing the methanol extracts of *S. mahagoni* leaves, its antioxidants activities and the phenols contents using the Gas Chromatography-Mass Spectrometry (GC-MS) in order to support its utilization in Indonesia.

Materials and Methods

Sample collection and extraction

The leaves sample, about 2 and 3 years old, of *S. mahagoni* were obtained from Temanggung, Central Java, Indonesia (Perhutani). These green leaves were collected from 3 different and then cut into small pieces. The actual fresh sample required (15 g) was extracted using *n*-hexane for three days at room temperature. The sample was then filtered using filter paper and evaporated using a rotary evaporator. The dried extract was then weighed to obtain the amount of *n*-hexane extract as a percentage of the fresh leaf sample. Subsequently, another sample was extracted with methanol using the former protocol.

Total phenol content (TPC)

The Folin-Ciocalteu method was used to determine the TPC of the sample. This involved the mixture of 2.5 ml of Folin-Ciocalteu phenol reagent (10 times dilution) and 0.5 ml of sample (1000 ppm) and incubated for 2 min at room temperature. Further, 2 ml of 7.5% aqueous sodium carbonate was added to the solution and then incubated for 30 min, also at room temperature. The sample absorbance was read at 765 nm and the results of TPC were expressed as gallic acid equivalents (mg GAE/g extract). The TPC measurement was conducted in three replications.

Total flavonoid content (TFC)

The aluminium chloride ($AlCl_3$) method, according to Brighente *et al.* (2007), was used to determine the sample TFC. This involved reaction between 2 ml of 1000 ppm sample and 2 ml of 2% $AlCl_3.6H_2O$ solution. This was allowed to stand for 1 h at 20 °C and the sample absorbance was read at 415 nm. The results from three replications were expressed in quercetin equivalents (mg QE/g extract).

GC-MS analysis

Prior to being subjected to GC-MS, the sample was methylated based on the method proposed by Jantan *et al.* (1999). This involved the mixing together of 1 ml 1000 ppm sample with 100 µl of tetramethyl ammonium hydroxide (TMAH). Then, 1 µl of the sample was injected to the GC-MS machine in three replications and data collected with a GCMS-QP 2010 (Shimadzu, Japan). The GC condition: Rtx-5MS capillary column (30 m x 0.25 mm I.D. and 0.25 µm; GL Sciences, Tokyo, Japan); detection temperature of 285 °C; column temperature from 70 °C (2 min) to 290 °C at 5 °C/min; injection temperature of 200 °C; acquisition mass range of 50-500 amu using helium as the gas carrier. Finally, the mass spectra of the sample was compared with the NIST library and the peak relative method was used for quantification of the compound. In addition, the minimum similarity index mass spectra between library and sample was 60%.

DPPH scavenging activity

The antioxidant activity assay was conducted in thrice measurement. This involved mixing 0.1 ml extract in methanol with 1000 ppm sample and then allowed to react with 3 ml of 0.1 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH), and then incubated for 30 min. Then, its absorbance was read at 517 nm and the antioxidant activity calculated using equation (1):

$$\text{DPPH scavenged (\%)} = 100 \times (Ao - A1) / Ao \qquad (1)$$

where Ao is absorbance of blank and A1 is absorbance of sample.

Statistical analysis

The results from the 2- and 3-year old leaves were subjected to independent t-test analysis with 95% confidence interval using SPSS 20 (IBM, USA). All assays were conducted in triplicates.

Chemicals

The following chemicals, TMAH, DPPH and gallic acid were purchased from Sigma-Aldrich (Germany).

Results and Discussion

Extractive content

The amount of methanol extract of *S. mahagoni* leaves ranged from 5.6 ± 0.36 to 7.0 ± 0.96 % (means ± standard deviation). Also, based on t-test, there was no significant difference in the amount of methanol extract between the 2- and 3-year-old leaves as $p > 0.81$, as shown in Table 1.

Table 1. Independent t-test between ages of *S. mahogani* leaves extracts

Parameter	T-test for equality of means	
	Sig. (2-tailed)	df
Extractive content	0.81ns	4
Total phenolic content	<0.01*	4
Total flavonoid content	<0.01*	4
DPPH radical scavenging activity	0.17ns	4
Phenolics group constituent	0.97ns	4
Fatty acids and hydrocarbons group constituent	0.63ns	4
Terpenoids group constituent	0.62ns	4

ns=not significant; *=significant at 0.05 level

Theoretically, polar solvents such as methanol dissolve phenolic compounds (Fengel and Wegener 1989; Sjöström 1993). However, in this experiment, the use of methanol was to identify the chemical properties of *S. mahagoni* leaves, as well as its antioxidant activity. Also, the amounts of methanol extract in this result were lower compared to the 90% ethanol extract of *S. mahogani* and *S. macrophylla* leaves from plants cultivated at the Zoological Garden, Giza, Egypt progeny (Mousa *et al.* 2014) and *Azadirachta indica* leaves from Botanical Survey of India (Alok *et al.* 2011). However, it was higher than the value of 80% aqueous ethanol extract (1.58 to 6.2 %) from *Azadirachta indica* from Kogi State, Nigeria (Raphael 2012).

Total phenol and flavonoid content

The total phenolic and flavonoid contents of the methanol extracts ranged from 36.4 ± 0.84 to 42.0 ± 0.18 mg GAE/g and 2.24 ± 0.15 to 18.55 ± 1.05 mg QE/g respectively of both dried extracts (means ± standard deviation). The t-test was conducted to observe the effects of tree ages and results showed that it has significant effects on the TPC and TFC of methanol extract. The TPC in 3-year-old leaves was over two folds higher than the 2-year-old samples. Similarly, the TFC of the 3-year-old was nine times compared with the 2-year-old leaves. This showed that the 3-year-old sample is a potential source of phenolic molecules for antioxidant, antimicrobial and antifungal activities.

This study also revealed that the extractive contents of the 2- and 3-years old leaves were not significantly different but the phenols contents differed significantly. In addition, the TPC of the leaves was lower compared with the methanol extract of *S. mahogani* seeds at 55 mg GAE /g dried extract

(Salleh *et al.* 2014). Therefore, in comparison, the TFC of *S. mahagoni* leaves in this experiment was in a lower concentration.

GC-MS analysis

The analysis of methanol extracts from the leaves of *S. mahagoni* using GC-MS is shown in Table 2 and the chromatogram in Fig. 1. Based on Table 1, there was no significant difference in the concentration of phenolics, fatty acids and hydrocarbons, and terpenoids in both the 2- and 3-year-old leaves. The leaves constituents are these three substances; phenolics, fatty acids and hydrocarbons, and terpenoids. These were dominated by fatty acids and hydrocarbons as well as terpenoids, while phenolics were found in low quantity. In addition, the ratio between terpenoids - fatty acids and hydrocarbons was almost 1:1 and the predominant compounds in fatty acids and hydrocarbons were palmitic and linolenic acid. However, squalene and α-tocopherol were the main components of terpenoids while 1-pyrrolidinebutanoic acid 4-methoxyphenyl ester was the highest in phenolics. Several components in this experiment showed a compatively lo similarity index level which suggests possibility assignments for other components. Therefore, standard components should be used to confirm those actual constituents in further works.

Table 2. GC-MS results of methanol extracts of *S. mahagoni* leaves

No	Ret. time (min)	Constituents	Concentration (% of dried extract) ± std. dev		Similarity index (%)
			2 years	3 years	
		Phenolics	**4.32 ± 0.91**	**4.39 ± 2.98**	
1	19.11	Disophenol	0.64 ± 0.31	1.79 ± 1.83	71
2	40.64	1-Pyrrolidinebutanoic acid 4-methoxyphenyl ester	1.92 ± 0.73	1.54 ± 0.66	62
3	40.90	1-Pyrrolidinebutanoic acid 4-methoxyphenyl ester isomer	1.76 ± 0.15	1.06 ± 0.93	62
		Fatty acids and hydrocarbons	**47.15 ± 4.08**	**44.65 ± 7.25**	
4	25.11	4-Undecene, 7-methyl	0.33 ± 0.33	0.62 ± 0.05	76
5	25.34	2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1,8-diol	2.57 ± 0.36	2.72 ± 1.18	79
6	25.85	1-Hexadecyne	3.08 ± 0.80	4.07 ± 0.79	89
7	25.99	Hexahydropseudoionone	0.84 ± 0.40	0.48 ± 0.42	89
8	26.29	Phytol, acetate	041 ± 0.10	0.51 ± 0.13	79
9	26.61	1-Octadecyne	1.04 ± 0.28	1.41 ± 0.16	86
10	27.40	Palmitic acid, methyl ester	5.76 ± 0.43	5.17 ± 0.91	94
11	28.15	Palmitic acid	13.35 ± 2.7	7.31 ± 6.46	91
12	30.20	Linolelaidic acid, methyl ester	2.30 ± 1.46	2.58 ± 0.78	92
13	30.29	12-Octadecenoic acid, methyl ester	1.69 ± 1.62	2.16 ± 0.17	93
14	30.32	Hexadecatrienoic acid, methyl ester	3.94 ± 0.88	3.50 ± 0.23	72
15	30.67	Stearic acid, methyl ester	1.43 ± 0.63	1.49 ± 0.42	89
16	30.93	Methyl 13,14-octadecadienoate	1.41 ± 1.18	1.62 ± 1.46	60
17	31.06	Linolenic acid	7.72 ± 1.17	10.09 ± 1.41	91
18	34.15	trimethyltridecyl)dihydro-2(3H)-furanone	0.78 ± 0.38	0.46 ± 0.40	84
19	43.62	1-Chloroeicosane	0.51 ± 0.57	0.47 ± 0.44	79
		Terpenoids	**39.67 ± 5.14**	**42.48 ± 7.49**	
20	40.22	Squalene	19.42 ± 5.55	21.77 ± 5.87	95
21	40.51	α-Farnesene	2.82 ± 3.47	1.67 ± 0.83	68
22	42.17	Stigmast-5-en-3-ol, oleate	0.60 ± 0.11	0.45 ± 0.16	60
23	44.68	α –Tocopherol	9.93 ± 1.34	12.74 ± 5.04	90
24	44.75	α-Tocopherolquinone	6.90 ± 0.12	5.85 ± 1.67	65

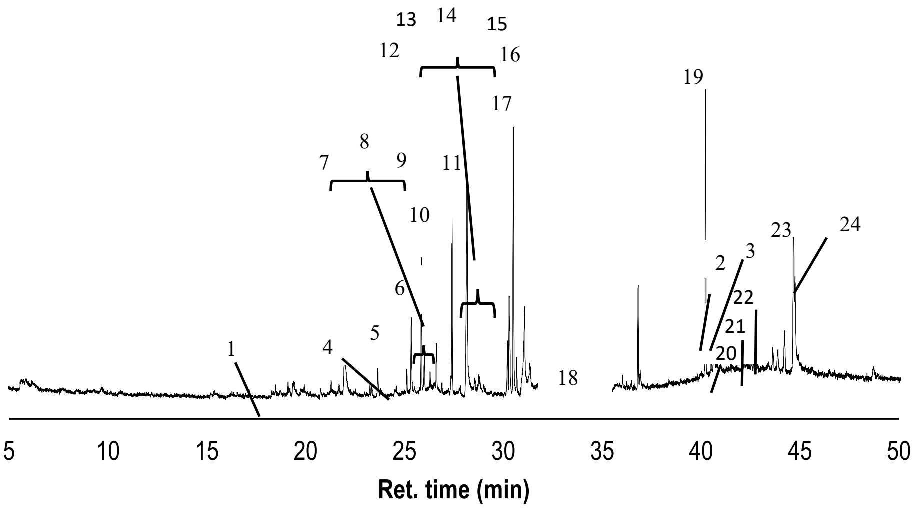

Figure 1. Chromatogram of GC-MS of methanol extracts of *S. mahagoni* leaves; 1. Disophenol, 2. 1-Pyrrolidinebutanoic acid 4-methoxyphenyl ester, 3. 1-Pyrrolidinebutanoic acid 4-methoxyphenyl ester isomer, 4. 4-Undecene, 7-methyl, 5. 2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1,8-diol, 6. 1-Hexadecyne, 7. Hexahydropseudoionone, 8. Phytol, acetate, 9. 1-Octadecyne, 10. Palmitic acid, methyl ester, 11. Palmitic acid, 12. Linolelaidic acid, methyl ester, 13. 12-Octadecenoic acid, methyl ester, 14. Hexadecatrienoic acid, methyl ester, 15. Stearic acid, methyl ester, 16. Methyl 13,14-octadecadienoate, 17. Linolenic acid, 18. trimethyltridecyl)dihydro-2(3H)-furanone, 19. 1-Chloroeicosane, 20. Squalene, 21. α-Farnesene, 22. Stigmast-5-en-3-ol, oleate, 23. α -Tocopherol, 24. α -Tocopherolquinone

Antioxidant activity

The antioxidant activity of methanol extracts from the leaves of *S. mahagoni* is shown in Fig. 2. The concentration of methanol extracts from each of 2- and 3-year-old leaves was 1000 ppm, with antioxidant values of 66.45 ± 1.85% and 77.59 ± 11.23% respectively (means ± standard deviation). For the purpose of comparison, the positive controls of gallic acid standard with concentration of 250 ppm resulted to antioxidant value of 93.14%. Based on the GC-MS results, the antioxidant activity of *S. mahagoni* leaves might be affected by the presence of these three compounds (phenolics, fatty acids and hydrocarbons, and terpenoids). Also, since the phenolic components of the leaves were in low concentration as shown in Table 2, this might be the cause of the low antioxidative activity values.

The antioxidant activity of *S. mahagoni* leaves was higher than previous study on other species of *Tylophora asthmatica* (Malathi *et al*. 2012), and *Actinodaphne madraspatana* (Suneetha *et al*. 2014), *Sesbania grandiflora* (Roy *et al*. 2014) and *Odina woodier* (Valli and Jeyalakshmi 2012) leaves. Based on the GC-MS analysis, the constituents of the leaf extracts such as phenolics, fatty acids and hydrocarbons, and terpenoids, might be responsible for the leaf's antioxidant activity. However, an earlier study by Mousa *et al*. (2014) showed that phenolic compounds and terpenoids were absent, but fatty acids and hydrocarbons were detected in *S. mahagoni* leaves. To a large extent, the presence of phenolic compounds and terpenoids was due to methylation process during the GC-MS experiment, as some compounds were methylated in order to be detected by the GC detector. Further analysis of the fatty acids and hydrocarbons, showed the presence of palmitic and stearic acid in the leaves of *S. mahagon* (Mousa *et al*. 2014). The study also showed that palmitic and linoleic acid were its major compounds, which was a little bit different from the results of this study, where the dominant compounds from fatty acid group were palmitic with linolenic acid.

In this study, linolenic acid was detected in the 2- and 3-year old leaves at 7.72 ± 1.17% and 10.09 ± 1.41%, respectively. Additionally, the contribution of unsaturated fatty acids to the antioxidant activity of the leaves was higher than that of phenolic compounds. However, there is still of last group of the constituents of *S. mahagoni* i.e terpenoids, with a concentration almost similar to fatty acids and hydrocarbons. Thus, the presence of these compounds in *S. mahagoni* leaves have considerable effect on its antioxidant activity. The terpenoids was dominated by squalene, followed by α –tocopherol and α-tocopherolquinone, these compounds were suggested to have strong antioxidant activity (Liebler and Burr 2000). In comparison, α–tocopherol also was contained in leaf of *Abutilon indicum* (Ramasubramaniaraja 2011). Furthermore, the antioxidant activity of these leaves might be linked with the phenols content. There was increase in the total phenolic and flavonoid content from the 2-year old leaves to the 3-year. The same increase was also observed in their antioxidant levels.

Figure 2. Antioxidant activity of methanol extracts of *S. mahagoni* leaves

Conclusions

Based on the results, the extractive content of the 3-year-old was lower than the 2-year-old leaves. However, the phenols contents and antioxidant activity were more in the 3-year compared with the 2-year old leaves. Additionally, 24 compounds were characterized by GC-MS, and were grouped into phenolics, fatty acids and hydrocarbons, and terpenoids. This study established that the antioxidant activity of the *S. mahagoni* leaves was as a result of its phenols contents. Furthermore, the antioxidant activity in the leaves were strongly affected by the presence of α –tocopherol and its derivative (α-tocopherolquinone), as the high level of these compounds make the leaves a potential source antioxidant agents and vitamin E.

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Alkaline Pulping of Red Meranti (*Shorea selanica* Blume)

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Abstract

The suitability for papermaking of red meranti (*Shorea selanica* Blume) wood for three alkaline pulping processes i.e soda, soda-anthraquinone (AQ), and kraft was studied. The fiber morphology and chemical properties were also examined. Cooks were made for 20% (as Na_2O) activate alkali. The resulting pulp and paper properties were investigated. The basic density of red meranti was 0.42 g/cm^3 and can be classified to be of medium density. The fibre proportion (67.14%) and fibre length (1.07 mm) of red meranti in this study were within the range of tropical hardwoods. The derived values for Runkel ratio, slender ratio, and flexibility coefficient were 0.54, 54.93, and 0.62, respectively. Furthermore, total extractives, lignin, and cellulose contents as well as solubility in 1% NaOH were 5.17%, 31.05%, 45.20%, and 26.02%, respectively. The alkaline pulps showed low kappa number (16~22) and reject level (0.5~1.5%) with the best results for screened yield (47.41%) being achieved in soda pulping. With regard to strength and optical properties of the paper hand-sheets, soda-AQ pulping showed the highest value in burst index (2.36 KPa m^2/g), tear index (8.47 $\pm$ 1.13 mNm^2/g), and brightness (19.81%), whereas kraft pulping gave the best result in tensile index (28.39 Nm/g). The comparatively low values of yield and strength properties in kraft pulping might be due to overcooking in this experiment.

Keywords: red meranti, pulp yield, kappa number, strength properties, brightness.

Introduction

The escalation of paper consumption has prompted investigations into the potential of fast-growing species as raw material for the pulp and paper industry. Some dipterocarps species have been reported to have comparatively high mean annual diameter increment (1.16~1.30 cm/year) and considered to be high potential for rehabilitation of the logged-over area in a large scale (Ådjers *et al.* 1995; Shono *et al.* 2007; Hassan *et al.* 2007). *Shorea selanica* Blume is a fast-growing tree which is grown commercially for the production of hardwood timber. Along with *S. leprosula* and *S. ovalis* from Kalimantan, *Shorea selanica* from Maluku are classified as ‘red meranti' timber and as 'Critically Endangered' in the IUCN Red List of Threatened Species (Ashton 2011). Therefore, establishment of fast growing species plantations with high productivity such as the line planting of red meranti with intensive silviculture in tropical rain forest has been intensified (Na’iem and Widiyatno 2012).

Kraft (sulphate) pulping is the dominant chemical pulping process used for the production of pulp fibres from various lignocellulosic materials. On the other hand, soda-anthraquinone pulping has environmental and economic advantages. Since no sulfur compounds are used, there is no such unpleasant smell generation. Furthermore, it increases the pulp yield and requires less cooking duration to obtain the same pulp quality and high yield values (Francis *et al.* 2006). Due to the effectivity, the anthraquinone addition may be applied to any raw – wood or non-wood – material (Biswas *et al*. 2011; Feria *et al.* 2012; Garcia *et al.* 2012; Gonzales *et al.* 2013).

In recent years, the attention has been focused on pulping of several hardwoods as alternatives to *A. mangium* in Indonesia (Lukmandaru *et al* 2002; Yahya *et al*. 2010; Theo 2011). However, there is still little knowledge on pulping of new potential species. To the best of our knowledge, no study has been conducted to investigate the pulping of *Shorea selanica*. One paper reported the pulping potentials and occurrence of pith problems of other shorea species i.e. *Shorea albida, Shorea richetia*, and *Shorea polita* (Su *et al*. 1992) with varied results. Therefore, the present work aimed at evaluating the wood properties and the alkaline pulp produced from red meranti wood and comparing it with those from *Acacia mangium* wood. The kraft, soda and soda-anthraquinone (AQ) pulping of the wood were conducted.

Materials and Methods

Sampling

Wood samples (Fig. 1) were obtained from a single tree (20 years) grown at the campus yard of Faculty of Forestry, Universitas Gadjah Mada, Jogjakarta. Stem disks (diameter of 20~30 cm, heartwood proportion ca. 27%) were cut from the base, center, and top parts at a certain height. The wood samples from each tree height were chipped (3 cm × 2 cm × 2-3 mm) manually, mixed, and air-dried.

Figure1. Cross section of the red meranti stem

Fibre Morphology

Cross sections (12 μm thick) were microtomed from wood block (American Optical Corp., New York, USA) and stained with a 0.1% solution of safranin (WAKO Pure Chemical Industries, Osaka, Japan), and mounted in glass slides. The cross-sectional images were captured under a light microscope (Olympus BX 51; Olympus Corporation; Japan) with a digital camera (Olympus DP 70; Olympus Corporation; Japan) and converted to digital format. Proportion of wood cell types, which are vessel, fibre, ray parenchyma and axial parenchyma, were measured in percentage. Fibre morphology, which comprised fibre diameter, fibre lumen diameter, and fibre wall thickness according to IAWA (1989), was also measured. Lengths of wood fibres were measured with image-analysis software (Image pro Plus). The cell length of 100 fibers, macerated from small sticks with Franklin's solution, was measured by a digitizer (Olympus DP 70; Olympus Corporation; Japan) coupled to a light microscope (Olympus BX 51; Olympus Corporation; Japan). The cell morphologies of 100 randomly selected fibers were measured by image-processing software (Image pro Plus) according to IAWA (1989).

The derived fibre properties are defined as follows (Yahya *et al*. 2010 and the literatures cited therein):

Runkel ratio = double fibre wall thickness / fibre lumen diameter ··· (1)
Slenderness ratio = fibre length / fibre diameter ··········· (2)
Flexibility coefficient = fibre lumen diameter/ fibre diameter ··· (3)

Basic Density

The basic density was determined as the ratio of oven-dry weight to green volume as determined by the water displacement method.

Chemical Analysis

Extractives content was determined by extraction sequences with ethanol/toluene (1/2, v/v) (ASTM D1107 - 96) and hot water (ASTM D1110 - 84). The acid-insoluble Klason lignin (SNI 0492:2008), holocellulose (Wise's chlorite acid method, Browning 1967), and α-cellulose (NaOH extraction, Rowell *et al.* 2005) were determined in extractive-free wood. Hemicellulose content was determined by subtracting holocellulose from the cellulose contents. Ash content and solubility values in 1% NaOH were determined according to SNI 14-1031-1989 and ASTM D 1109 – 84 (2001), respectively.

Alkaline Pulping of Wood

Alkaline pulping of red meranti wood (250 g, oven-dried chip) was performed using a digester. The following kraft, soda, and soda-AQ cooking conditions were used: maximum cooking temperature of 170 °C, time required to reach the targeted maximum temperature of 60 min, cooking time at maximum temperature of 120 min, and liquor-to-wood ratio of 4 to 1. The active alkali was 20% as Na_2O on oven-dry wood in all cases. Sulfidity of 25% was used in the kraft pulping, whereas the AQ percentage was set 0.1% based on the oven-dried chip in the case of soda-AQ pulping. A total of two replications were then made for each treatment and the average reading was taken. Subsequently, the pulps were washed, screened and processed using fibre sorter equipped with a 100 mesh slot screen. Pulp yields and rejects were determined based on the oven-dry weight of wood chips initially charged to the digester. Pulps then were evaluated for kappa number (SNI ISO 302, 2014). Pulpability factor was calculated by dividing screened pulp yield by the kappa number (Little *et al.* 2003).

Beating and Physic-mechanical Properties of Pulp

Pulp samples were withdrawn on the Niagara beater after beating for 200~300 ml Canadian Standard Freeness (CSF) testing (SNI ISO 5267-2-2010) and hand-sheet formation (SNI ISO 5269-1-2012). Hand-sheets, each weighing 80 g/m^2, were made in a laboratory-scale sheet (diameter of 15.9 cm). Hand-sheets were conditioned (23 ± 2 °C and 50 ± 2% relative humidity) and tested for tear (SNI 14-0436-1989), tensile (SNI 14-0437-1989-A), burst (SNI ISO 2758-2011), brightness (SNI ISO 2470-1-2014), and opacity (ISO 2471, 2008).

Results and Discussion

Physical, Morphological, and Chemical Properties

The pulp and paper properties of wood species depend considerably upon their basic properties. Therefore, physical properties (Table 1) and the chemical composition (Table 2) of red meranti were determined to better understand the pulp and paper properties. The basic density of wood affects the pulp production. Pulpwood with a basic density greater than 0.60 g/cm^3 is not recommended (Little *et al.* 2003). Lower wood density certainly gives less pulp production per digester. Table 1 shows data on the properties of red meranti and compares the data with those of *A. mangium* (7 years, Yahya *et al*. 2010). The basic density of red meranti was 0.42 g/cm^3, which was slightly lower than that of *A. mangium* (Table 1), and the wood can be classified as medium density wood. It is assumed that the basic density of this sample was good for kraft pulping.

As observed under light microscope (Figs. 2), several cell types could be distinguished (fibres, rays, parenchyma cells, and vessel elements). Based on the measurement of various cell areas, the fibres of red meranti composed 67.14% of its total cells. The fibres of red meranti are long and cylindrical. The higher proportion of fibre and lower proportion ray (7.14%) and parenchyma (7.85%) tissues compared *to A.* mangium will give an advantage to produce higher yield. Fibre proportion and tear factor or folding endurance were positively correlated (Ona *et al.* 2001). However, the higher vessel proportion technically potentially causes vessel picking in paper manufacturing.

Longer fibre length, higher flexibility coefficient and/or lower wall-to-lumen ratio in wood are important aspects in pulping and papermaking (Xu *et al.* 2006). The fibre length of red meranti in this study was 1.07 mm, which was in the range of fibre length of most tropical hardwoods (Fengel and Wegener 1984). The fibre diameter was within medium range (21.16 µm). The fibre length, fibre wall thickness, and fibre diameter of red meranti were more that those of *A. mangium*. A positive correlation was found between fibre length and burst strength (Ona *et al.* 2001), tear strength (Shmulsky and Jones 2011) and folding endurance (Ona *et al.* 2001). Thick-walled fibres produce paper with low burst and tensile strength (Shmulsky and Jones 2011).

Table 1. The basic density, fibre morphological characteristics, and derived values of red meranti as compared to *A. mangium*

Physical properties	*Shorea selanica*	*Acacia mangium* [a]
Basic density (g/cm^3)	0.42	0.46
Cell Proportion		
Fibre (%)	67.14	62.46
Ray (%)	7.14	9.77
Parenchyma (%)	7.85	15.66
Vessel (%)	17.85	12.11
Fibre Dimension		
Fibre length (µm)	1070 ± 5.44	982
Fibre lumen diameter (µm)	13.20 ± 3.88	14.29
Fibre wall thickness (µm)	3.78 ± 0.45	2.55
Fibre diameter (µm)	21.16 ± 3.24	19.39
Derived values		
Runkel ratio	0.54 ± 0.12	0.37
Slenderness ratio	54.93 ± 8.55	51.29
Flexibility coefficient	0.62 ± 0.10	0.73

Remark : [a] Yahya *et al.* (2010), 7 year-old trees.

A direct correlation exists between fibre morphology and paper properties (Ververis *et al.* 2004). The slenderness and Runkel ratio values of red meranti were higher but its flexibility coefficient was lower than *A. mangium*'s. Raw materials with low Runkel ratio are preferred for paper making (Ohshima *et al.* 2005). It indicates the ability to collapse easily and form good fibre-to-fibre bonding. There is a positive correlation between slenderness ratio and folding endurance (Ona *et al.* 2001). Less flexible fibers do not produce large contact areas for fiber-to-fiber bonding. Its high flexibility is expected to have a positive effect on tensile and bursting strengths as well as on folding endurance (Xu *et al.* 2006). The results of the derived values in Table 1 indicate that red meranti sample fall within desirable ratios for paper making.

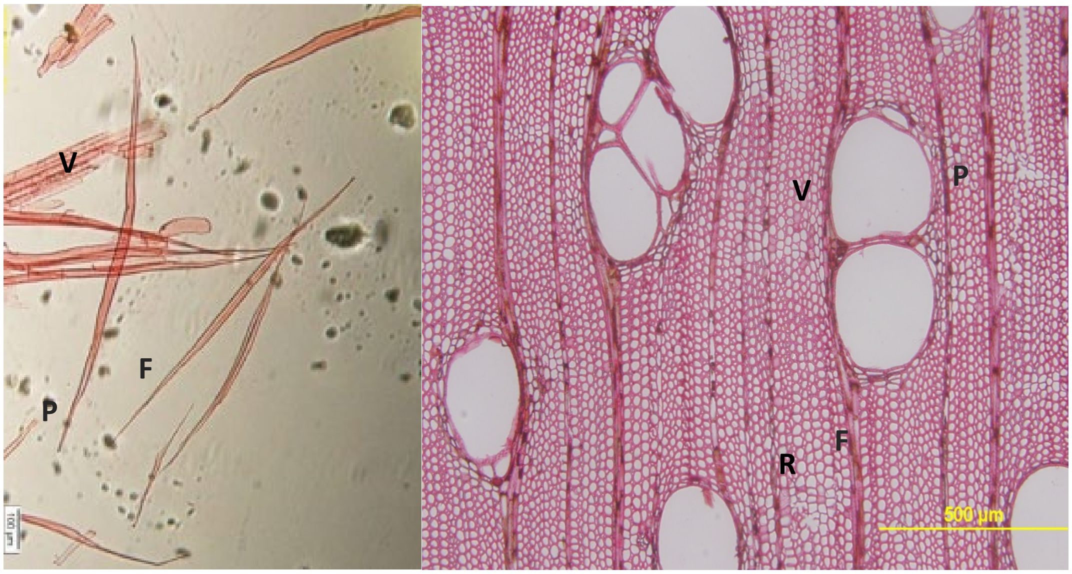

Figure 2. Cells from the *Shorea selanica* (100×). The different cell types identified in the pulps are F: fibre; R: ray, V: vessel element; P: parenchyma.

The lignin and cellulose contents in red meranti were almost similar to those in *A. mangium* (Table 1). The ethanol-toluene extract of this species was lower than that of *A. mangium*. Low extractive contents in wood are desirable for pulping, bleaching, and paper making. Total extractive content (the sum of ethanol-toluene extractive content and hot-water solubility) was about 5.17%. However, red meranti also had high level of solubility in 1% NaOH (26.02%). High 1% alkali solubility may be attributed to higher amount low molecular weight polysaccharides. The comparatively high 1% alkali solubility value may result in lower pulp yield from red meranti in the present investigation. The hemicellulose in red meranti was lower than that in *A. mangium* (Table 1). The lower hemicellulose in red meranti in this investigation may be the basis of higher pulp yield. The ash content (0.7%) was much lower than that of usual tropical species (1-3%) (Khristova *et al.*1997).

Table 2. The chemical composition of red meranti as compared to *A. mangium*

Chemical properties	*Shorea selanica*	*Acacia mangium* [a]
Ethanol-toluene extractive content (%)	3.74	5.38
Hot-water solubility (%)	1.97	-
Holocellulose (%)	69.83	80.43
α-Cellulose (%)	45.20	45.71
Hemicellulose (%)	24.65	34.72
Lignin (%)	31.05	31.30
Ash (%)	0.73	-
Solubility in 1% NaOH (%)	26.02	-

Remark : [a] Yahya *et al*. (2010), 7 year-old trees

Pulping and Pulp Properties

Red meranti was pulped in soda, soda-AQ, and kraft pulping processes (Table 3). In all processes, the kappa number of pulp obtained was low (15.11~17.72). In 2 h of cooking, pulp yield obtained was 39.94%~47.41%. The highest screened yield (47.41%) and moderate kappa number (20.52) were obtained in soda pulping process, which were better than those of *A. mangium*. In the similar cooking condition, soda-AQ pulp had lower screened yield and higher kappa number than soda pulp. However, it is clearly seen that the kraft process showed its superiority for delignification over soda and soda-AQ processes with kappa number being 16.17 and reject being 0.56% but low in screened yield (39.94%).

Chemical additives can be used in pulping to reduce reactions of polysaccharides or increase reactivity of lignin. AQ additionaly accelerates the delignification rate of pulping (Dimmel *et al.* 2003). Red meranti showed similar kappa number (about 20~22) in the soda and soda-AQ pulping processes, but the yield was higher in soda pulping process. In an earlier report, delignification was accelerated by AQ addition in soda liquor (Parthasarathy *et al.* 1995). Similar trend was also observed in *A. nilotica* pulping (Khristova and Karar 1999). It means that addition of AQ in soda liquor during red meranti pulping did not increased selectivity of pulping. Thus, another approach for different concentrations should be attempted.

The higher values of pulpability factor (greater than 2.34) is recommended with the ideal basic density between 0.46 and 0.52 g/cm^3 (Gardner *et al.* 2001). Kraft process showed pulpability factor greater than 2.34, which indicate good pulpwood quality without having to do multiple cooks and interpolate to the desired 20 Kappa number. The values of red meranti were higher than those of *A. mangium* (2.41).

In this investigation, kraft pulp yield of red meranti was lower than that of *A. mangium*. Lower pulp yield in the present investigation may also be caused by high active alkali (20%) or sulfidity (25%) used in this experiment. The chips may be overcooked because of the use of more severe cooking conditions. A high 1% alkali solubility value, may be another reason of lower pulp yield.

Beatability of pulps is significant variable for the energy consumption of mills and generally depends on the chemical composition of pulps. The highest initial beating degree was observed in soda-AQ samples (686 ml CSF), which indicates more energy consumption. It indicates higher lignin content as indicated by higher kappa number (21.92) and lower hemicellulose content.

Table 3. The pulp properties of red meranti alkaline pulp obtained at 20% active alkali

Pulp Properties	Soda	Soda- AQ	Kraft	*Acacia mangium* -kraft [a]
Screened yield (%)	47.41 ± 1.90	45.75 ± 3.44	39.94 ± 0.21	45.02
Reject (%)	1.51 ± 0.90	1.50 ± 1.17	0.56 ± 0.08	3.54
Kappa number	20.52 ± 0.79	21.92 ± 2.44	16.17 ± 0.44	18.61
Pulpability factor	2.31	2.08	2.47	2.41
Initial beating degree (ml CSF)	630.0 ± 14.1	686.6 ± 11.5	637.0 ± 77.8	-

Remark : [a] Haroen and Dimyati (2006) . Pulping condition : active alkali of 17%, sulifidity of 25%, maximum temperature 165 ^{0}C, duration of 3.5 h

In order to examine the paper properties of red meranti, standard paper hand-sheets were produced from their pulps. The results of physical paper properties obtained at different alkaline processes are shown in Table 4. For the purpose of comparison, properties of hand-sheets from pulps of mangium pulp-woods obtained through kraft process (Haroen and Dimyati 2006) are also included in the Table. In an earlier study, the burst index produced from kraft-AQ gave lower values compared to those of soda-AQ in orange tree wood (Gonzales *et al.* 2013). Kraft pulp of *Acacia auriculiformis* showed slightly better tensile index than the soda pulp did and showed lower burst index than soda and soda-AQ pulps did (Jahan *et al*. 2009). In addition, kraft pulp had higher tear index than soda and soda-AQ pulp at higher beating degree. In this experiment, kraft pulping process showed slightly better tensile index compared to soda and soda-AQ processes. Furthermore, strength properties (burst and tear indices) and brightness level were improved with AQ addition. Kraft pulp showed higher tear index level than the soda pulp. Another paper demonstrated that the soda pulp of non-wood bagasse has better tensile and burst strength than the soda-AQ pulp owing to lower degree of delignification and higher pulp viscosity (Hedjazi *et al.* 2008). In kenaf pulping, kraft and kraft-AQ pulping processes produced slightly lower or comparable quality pulp than soda-AQ pulping process (Ang *et al.* 2010). Those varied patterns might be due to the different nature of raw materials and pulping conditions.

Brightness level has linear negative correlation with kappa number (Gulsoy and Tufek 2013). The comparatively high brightness value of soda-AQ pulp might be because residual lignin containing highly colored chromophoric groups were more intensively reduced (Serkov and Alen 2004). Although almost in similar levels, the highest opacity (99.66%) was obtained in soda pulping process. The high opacity values indicate the high the light scattering coefficient of the pulps which increases the fiber-air interface number. This behaviour is probably due to differences between pulps at physical and chemical levels (Gonzales *et al.* 2013).

Compared to kraft *A. mangium* pulp, the red meranti kraft pulp showed considerably lower tensile and burst indices. Technically, tensile and burst indices depend on bonding ability of fibers. It indicates that red meranti paper does not produce large contact areas for fiber-to-fiber bonding or has less flexible fibres. Based on fibre properties, lower burst and tensile indices of red meranti pulp could be explained by lower fiber flexibility or bonding ability (Xu *et al.* 2006) and higher Runkel ratio (Ohshima *et al.* 2005) of red meranti fibers (Table 2). Another explanation could be that severe cooking kraft conditions in this experiment caused polysaccharides degradation. More moderate cooking kraft conditions should be performed to improve the sheet physical properties in the future work. The tear index of red meranti was almost similar to that of *A. mangium*. It could be attributed to longer, (Shmulsky and Jones 2011), thicker fibres (Scott *et al.* 1995) and also its higher slenderness ratio (Ona *et al.* 2001) (Table 2). Brightness of red meranti pulp gave lower values compared to that of *A. mangium*. It indicates more residual lignin containing highly colored chromophoric groups on fiber surfaces (Serkov and Alen 2004). Based on national standard (SNI) for leaf (hardwood) bleached kraft, only tear index and opacity of red meranti pulp met the requirements (Table 4). This suggests that red meranti kraft pulp could be a potential reinforcement component in products based on mechanical pulp, such as newsprint. Furthermore, it will be necessary also to evaluate the pulp properties of *S. selonica* wood at the harvest age (5-6 years) from industrial plantation forests.

Table 4. The paper properties of red meranti alkaline pulp obtained at 20% active alkali

Physical Properties	Soda	Soda-AQ	Kraft	*Acacia mangium* -kraft [a]	SNI [b]
Tensile index (Nm/g)	27.47 ± 2.10	27.83 ± 1.19	28.39 ± 0.28	78.75	45
Burst index (KPa m^2/g)	2.18 ± 0.04	2.36 ± 0.13	1.98 ± 0.34	6.58	2.5
Tear index (mNm^2/g)	4.79 ± 0.75	8.47 ± 1.13	6.82 ± 0.47	6.94	5.5
Brightness (%)	17.89 ± 0.48	19.81 ± 0.99	17.25 ± 0.84	46.98	-
Opacity (%)	99.66 ± 0.19	98.20 ± 1.16	99.38 ± 0.10	-	80-90

Remark : [a] Haroen and Dimyati (2006) , [b] Indonesia National Standard (SNI 6107, 2009)

Conclusions

The physical, morphological, and chemical properties of red meranti were evaluated in terms of its suitability for papermaking. The basic density, fibre proportion, and fibre length of red meranti in this study were within the range of those of tropical hardwoods. The lignin content in red meranti (and α-cellulose) was almost similar to that in *Acacia mangium*. The high level of total extractive content and high solubility in 1% NaOH of red meranti would potentially reduce the pulp yield. Soda, soda-AQ, and kraft pulping processes were studied. The highest screened yield was obtained in soda pulping. Kraft pulping showed the lowest kappa number and reject. Acceptable pulp yields were obtained at cooking in soda and soda-AQ pulping processes. Compared to soda and soda-AQ pulping processes, kraft pulping process gave slightly better tensile index whereas soda-AQ pulping process produced the highest value in tear index and burst index. Kraft pulp of red meranti showed considerably lower tensile and burst indices than the kraft pulp of *A. mangium*, which was probably due to higher concentration of chemicals during the cooking. However, soda-AQ pulp of red meranti produced better tear index compared to kraft pulp of *A. mangium.*

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Pulp and Paper Characteristics of Five Lesser-known Species in Kalimantan: Effects of Re-beating

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Abstract

Five lesser-known species from natural forests in Central Kalimantan, viz., cempaka (*Michelia champaca* Linn), mentawa (*Artocarpus rigidus* Blume), menjalin (*Xanthophyllum excelsum* Miq.), kempili (*Lithocarpus* elegans (Blume) Hatus. Ex Soepadmo), and sempori (*Dillenia* sp.) were evaluated in the laboratory for their specific gravity, fiber morphology, pulping and papermaking properties. In addition, their properties after three-phase beating were also evaluated from a recycled paper point of view. The specific gravity and fiber length range were 0.58~0.68 and 1239~2479 µm, respectively. The highest value in specific gravity was observed in menjalin wood, while the longest fiber was observed in sempori. Kraft pulping with 14% active alkali (as Na_2O), 23% sulfidity, 2 h at the maximum temperature showed that the highest screened yield was determined in cempaka wood (44.29%) with a kappa number of 17.6. The freeness ranges of unbeaten pulp were 675~780m mL CSF. The freeness ranges of 1st, 2nd, and 3rd beating were 539~630 mL, 235~275 mL, and 220~230 mL CSF, respectively. The 1st beating exhibited the best mechanical properties. Among the species, cempaka, kempili, and mentawa showed comparatively high tensile (57~60 Nm/g) and burst index (2.6~3.4 KPa m^2/g), whereas the highest value for tear index (5.02 mNm^2/g) was observed in sempori. A considerable decrease in fiber length, slenderness ratio, and mechanical properties of the paper was observed with an increased beating number. These findings suggest that cutting the fibers or decreasing the slenderness ratio was the main factor causing the strength to decrease.

Keywords: beating degree, secondary fiber, kraft pulping, pulp wood, fiber morphology.

Introduction

The growing consumption of pulp and paper in Indonesia relies mainly on the exploitation of forests for pulp wood and the recycling of fibres. In 2020, Indonesia is projected to produce 20.4 million metric tons of pulp and 19.8 million metric tons of paper (APKI 2014). Globally, recycled paper is becoming an increasingly important fiber source for the pulp and paper industry. Furthermore, the annual consumption of recycled paper (>120 million metric tons) has surpassed that of paper from non-wood sources (Henriksson *et al.* 2009). Increasing the use of lesser-known species and recycled fibers by rationalizing their valorization could allow the conservation of forest resources and bring new properties to wood-based products considering the specific properties of these fibers.

Cempaka (*Michelia champaca* Linn), mentawa (*Artocarpus rigidus* Blume), menjalin (*Xanthophyllum excelsum* Miq.), kempili (*Lithocarpus* elegans (Blume) Hatus. Ex Soepadmo), and sempori (*Dillenia* sp.) are lesser-known native tree species in Central Kalimantan, Indonesia. These species are naturally distributed and abundant in natural forests in Kalimantan. In addition, those species are also planted in other islands in Indonesia. To the best of our knowledge, no information has been published on the wood and pulp properties of these species. Studies of these properties are necessary for the improvement of the availability of pulp raw materials.

Cellulosic fibers obtained from pulping processes must be subjected to physical treatment in order to render them useful for making paper with acceptable properties (Mutje´a *et al.* 2005). These properties can be significantly improved by mechanical treatment of the fibers, a process known as beating or refining. Beating of pulp is a mechanical treatment that causes physicochemical changes in fibers by external and internal fibrillation of the cell wall (Emerton 1957). Beating or refining improves not only the fiber properties but also the paper properties. In some cases, the aim is to shorten fibers that are too long for good sheet formation or to develop properties such as absorbency, porosity, or optical properties for a certain paper grade (Lumiainen 2000). This process is the most energy-intensive in pulp and paper production. However, beating has undesirable effects, such as fiber shortening and the creation of axial micro compressions in the fiber wall (Rosli *et al*. 2011). Some factors, such as the extent of their mechanical and chemical damage and the length of the fibers, affect the strength of paper sheets (Levlin and Soderhjelm 1999). Therefore, it is necessary to study this negative influence with respect to recycled fibers.

In order to obtain accurate data to improve the quality of fibers from the five lesser-known species mentioned above, the goals of the research presently conducted were: (a) to describe the specific gravity and morphology in the wood fiber, (b) to describe the pulp properties from kraft pulping, (c) to discover the beating behaviour and mechanical properties of the paper, and (d) to discover the effect of re-beating on fiber morphology and mechanical properties of the paper. Energy savings and the desired paper properties can be achieved by applying proper beating process conditions, and improper beating cannot be compensated for in the subsequent processing steps.

Materials and Methods

Sample Preparation

Wood samples of cempaka, mentawa, menjalin, kempili, and sempori were collected from the lower part of trees (unknown age, ± 1.3 m above ground) in the natural forest concession managed by PT. Sari Bumi Kusuma, Central Kalimantan. The wood disc was then cut into a strip of 5 cm thickness. The wood strip was converted into chips (3 cm × 2 cm × 2-3 mm) for pulping and blocks (2 cm × 2 cm × 2 cm). The blocks were randomly selected for specific gravity and fiber dimension measurements. The chemicals used for pulping were NaOH and Na_2S (technical grade) whereas for kappa number determination were $KMnO_4$, KI, H_2SO_4, starch, $Na_2S_2O_3$ (PA grade).

Fiber Morphology and Specific Gravity

Fiber dimensions were analyzed from square wood samples measuring 1 mm x 1 mm x 20 mm (tangential, radial and longitudinal directions). The wood samples were macerated with the mixed solution of glacial acetic acid and hydrogen peroxide (1:20, PA grade) and heated at 100°C for more than 4 hours. The stained wood fibers were then placed on deck-glass. The wood fibers of each species were photographed with an optical light microscope (OLM, Olympus BX 51 DP 72, Japan). The dimensions, including fiber length (4 × magnification), cell diameter, cell wall thickness, and lumen diameter (40 × magnification) of wood fibers were examined and recorded (Image Pro Plus V.4.5 software). Determination of the percentage of intact and broken fibers (damage) was performed by 4 × magnification of fiber dimension specimens. Green specific gravity (wet volume/oven-dry weight) was prepared and measured according to British Standard No. 373 (1957).

Runkel ratio, slenderness ratio, coefficient of rigidity, Luce's shape factor, and flexibility coefficient were calculated using the following equations (Tamolang and Wangaard 1961; Dinwoodie 1965; Ogbonnaya *et al.* 1992):

Runkel ratio = Double fiber cell wall thickness/Fiber lumen diameter *(1)*
Coefficient of rigidity = Fiber wall thickness/Fiber diameter *(2)*
Slenderness ratio = Fiber length/Fiber diameter *(3)*
Flexibility coefficient = Fiber lumen diameter/Fiber diameter *(4)*

Fiber dimensional changes after beating were calculated using the following equations:

Fiber dimensional reduction = (Initial dimension from macerated samples – Post-beating dimension) × 100 *(5)*
Fiber dimensional addition = (Post-beating dimension - Initial dimension from macerated samples) × 100 *(6)*

Pulping

Preparation of kraft pulps consisted of white liquor (NaOH and Na_2S). Kraft pulps were processed in a 5-liter KRK laboratory rotary autoclave. The raw material (300 g, oven dry weight) was cooked in the autoclave with a liquor-to-wood ratio of 4:1. The pulp conditions were active alkali 14% as Na_2O and sulfidity of 23%. Pulps were processed for 120 min at 170~180°C with time to maximum temperature was 60 min. The target of kappa number was 18. The pulps were dispersed with a standard pulp disintegrator. Then, the pulps were placed in a No. 100 mesh screen to separate the screened pulps and reject them. The kappa numbers of the screened pulps were determined according to standard test methods SNI ISO 302 (2014).

Niagara Beating

Niagara beating is performed in a batch with a low-consistency pulp. The beating is looped around a well and forced between a rotor bar and a loaded bedplate to generate a mechanical shearing action. Kraft hardwood pulps were loaded into the Niagara beater (KRK, Japan) and refined at 0.3% consistency with a 1 kg load for the first, second, and third beatings. The first beating was conducted for 5 minutes, whereas the second and the third beatings were conducted for another 5 minutes at each phase. Samples for fiber measurement and papermaking were taken from the laboratory beater at the three-phase beating or beating number. The drainage capabilities of the unbeaten and beaten pulps were measured with a KRK Canadian Standard Freeness (CSF) tester according to SNI ISO 5264-1-2011.

Dry Strength Evaluation

Hand sheets of 80 g/m^2 were produced on a sheet former (diameter of 15.9 cm) according to SNI ISO 5269-1-2012. The standard hand sheets were conditioned at 23±2°C and 50±2% relative humidity. The following tests for dry strength (mechanical) properties were determined using standard SNI ISO methods: (i) Tensile strength index, SNI 14-0437-1989-A; (ii) Burst strength index, SNI ISO 2758-2011; (iv) Tear strength index, SNI 14-0436-1989. Strength reduction in the 2nd and 3rd beatings was calculated using the following equations:

Strength reduction = (Strength in 1st beating – Strength in the 2nd or 3r beating) × 100
(7)

Results and Discussion

Specific Gravity and Fibre Morphology

Table 1 shows the mean value of the measured specific gravity of these species from the minimum to the maximum values and their fiber dimensions. The maximum specific gravity value was observed in menjalin (0.68) while the minimum was in sempori wood (0.58). This specific gravity ranges are slightly higher compared to commonly specific gravity for pulp and paper purposes i.e., 0.35~0.65 (Miranda *et al*. 2012). Among five wood species, sempori has the longest fiber length (2479 µm) followed by menjalin (1587 µm). Kempili has the highest value of fiber diameter, fiber lumen diameter, and fiber wall thickness. The correlation between specific gravity and cell wall thickness was unclear among those species. Menjalin had the highest specific gravity level but the cell wall thickness was lower compared to the other species. It is assumed that extractives content also affects the value of specific gravity. With regard to derived values, slenderness ratio of sempori was the highest (111.9) whereas the highest flexibility coefficient (0.70) was found in mentawa. Furthermore, the lowest value of Runkel ratio (0.58) was found in mentawa and sempori, whereas the lowest coefficient rigidity (0.19) was calculated in menjalin and sempori.

A positive correlation was found between fiber length and burst strength (Ona *et al*. 2001) as well as between fiber length and tear strength (Shmulsky and Jones 2011). Thick-walled fibers are not expected because it will produce paper with low burst and tensile strength (Shmulsky and Jones 2011). Furthermore, the Runkel ratio measures the suitability of fiber for paper production, the slenderness ratio (Peteri coefficient) measures the tear property of pulp in paper production, the flexibility coefficient measures the strength properties of paper, and the coefficient of rigidity measures the tensile strength of fiber (Yanez-Espinosa *et al.* 2001). On the basis of derived values, the relatively low values in Runkel ratio and coefficient rigidity, as well as high values in slenderness ratio suggests that sempori and mentawa wood would exhibit better paper mechanical properties.

Figure 1 shows the morphology of these species from the shortest fiber to the longest one, respectively. Each wood fiber species, exhibits specific characteristics. There were three fibers that possessed a distinct and short tail proportion in one end, i.e., kempili, menjalin, and sempori. Sempori has a long fiber, which is very easy to distinguish from others. This fiber shows a rather special characteristic because the size of the fiber is close to conifer, such as *Pinus* merkusii. Mentawa and cempaka showed more tapered tails on both ends.

Table 1. Specific gravity and morphology of wood fibers of five species from natural forest

No	Wood characteristics	Sempori	Cempaka	Mentawa	Kempili	Menjalin
1	Specific gravity	0.58	0.60	0.66	0.66	0.68
2	Fiber dimension					
	Fiber length (µm)	2479	1316	1230	1395	1587
	Fiber diameter (µm)	22.14	19.45	17.83	26.82	19.28
	Fiber lumen diameter (µm)	13.55	10.38	10.52	15.69	11.84
	Fiber wall thickness (µm)	4.29	4.53	3.65	5.56	3.72
3	Derived values					
	Runkel ratio	0.58	0.87	0.58	0.74	0.62
	Slenderness ratio	111.97	67.65	68.99	52.01	82.33
	Flexibility coefficient	0.65	0.53	0.70	0.55	0.61
	Coefficient of rigidity	0.19	0.23	0.20	0.20	0.19

Figure 1. Morphology of wood fibers of five species from natural forest. Left to right: mentawa (*Artocarpus rigidus* Blume), Cempaka (*Michelia champaca* Linn), kempili (*Lithocarpus* elegans (Blume) Hatus. Ex Soepadmo), menjalin (*Xanthophyllum excelsum* Miq.), and sempori (*Dillenia* sp.)

Pulp Properties

The pulp yield and kappa number of five species were measured to evaluate them as pulping raw materials. As shown in Table 2 (from the lowest specific gravity to the highest one), the minimum pulp screened yield was observed in mentawa pulp (24.39%) and sempori pulp (26.96), and the maximum value was observed in cempaka pulp (44.20%). Kempili, menjalin, and sempori showed high levels of rejects (40~44%). Mentawa showed the highest kappa number (19.44), whereas kempili showed the lowest (17.22) among these five species. Although the kappa number was mostly at the target value (18), only cempaka gave the yield exceeding 40%. The pulp yield obtained under these operating conditions indicates that the concentration of active alkali is probably too low to obtain commercial level of yield (45~50%). It was assumed that the wood with high value of specific gravity might need more chemical concentration in this experiment. Mentawa and sempori showed almost similar pulp yield but had huge difference in rejects.

High value of the specific gravity of the wood results in high consumption of chemicals (Gomide *et al.* 2010). Low density will result in a lower kraft yield (Haroen 2017). This pattern of pulp yield cannot be explained by the variation in specific gravity (Table 1). On the other hand, a higher amount of lignin results in lower pulp yield (Shmulsky and Jones 2011) as well as lower paper mechanical properties (Fengel and Wegener 1989). Therefore, another factor, such as the chemical properties of these species, should be investigated to explain the variation in yield or kappa number.

Table 2. Pulp properties after kraft cooking of five species from natural forest.

No	Species	Screened yield (%)	Reject (%)	Kappa Number
1	Sempori	26.96	42.90	18.50
2	Cempaka	44.20	6.13	17.60
3	Mentawa	24.39	25.59	19.47
4	Kempili	36.94	44.82	17.22
5	Menjalin	33.74	40.22	19.14

Beating Properties

Freeness tests are commonly used to measure the effects of beating in an indirect way. The pulp freeness was measured as Canadian Standard Freeness (CSF). As shown in Table 3, the degree of beating is presented as a function of the beating number applied. The freeness, which was principally influenced by fiber fibrillation and the number of fine elements, was 675~780 mL CSF on average for the unbeaten pulp. The average range values decreased until it reached 220~230 at the 3rd beating (Table 6). For unbeaten pulp, sempori and menjalin gave the highest value of CSF (780 and 750 ml CSF, respectively), whereas cempaka gave the lowest value (675 ml CSF). The low values indicate more water was retained on the fiber surfaces. It is expected that the long fiber length in sempori wood would give the comparatively high rate in the freeness test.

The effect of pulp refining on fiber properties is that fiber shortening produces small particles (shorter than 200 µm) (Tonoli *et al.* 2013). They are commonly referred to as fines, which also contain band-like materials from both the primary and secondary wall layers of the fibers (Somboon *et al.* 2007). These fines increase the water retention of the web, fill the gaps between fibers, and lead to a reduction in CSF data. The reduction in freeness is visible as a function of beating. As shown in Table 3, as the number of beatings was increased, the degree of beating was reduced. Kempili showed a considerable reduction in beating degree after re-beating among these species. Fibers with low intrinsic viscosity (lower molar mass of cellulose and hemicellulose) were more easily damaged during refining than fibers with high intrinsic viscosity (Molin *et al.* 2004). The comparatively low value of kappa number with lower lignin content (less than 25%) in kempili may make the fiber less coarse and less resistant to mechanical action. In the third beating, the beating degree levels among the observed species were in slight differences. This indicates that the need of energy is almost similar to achieving 220-230 in the 3rd beating. Technically, high dewatering is preferable, while the reduction in draining of the pulp caused by refining (measured by freeness) is not desirable in the papermaking process.

Table 3. Beating degree (mL CSF) of kraft pulps from five species grown in natural forest

No	Species	Unbeaten/ initial	1st	2nd	3rd
1	Sempori	780	660	255	225
2	Cempaka	675	550	275	220
3	Mentawa	710	550	255	225
4	Kempili	700	530	235	230
5	Menjalin	750	640	255	225

Effect of Re-beating on Fiber Morphology

Beating degree is one of the main indicators of pulp dewatering performance, which can comprehensively reflect the degree of fiber cutting, swelling, and fiber fibrillation (Shi and He 2008). By observing cell morphology, a larger proportion of fibers were damaged by the increasing number of beating or refining actions during the pulping process, which was performed to produce a strong fiber bonding and a good surface smoothness for paper sheet (Casey 1980; Smook 1989). Fibers were damaged after beating, and the amount of damaged fibers increased with the number of beating (Table 4). The highest proportion of damaged fiber in the 3rd beating was observed in sempori samples, whereas the least was observed in kempili. In addition, sempori wood had the highest value of damaged fibers in each phase of beating, whereas kempili had the lowest damage in the 2nd and 3rd beatings. Based on the values of the first beating, mentawa, kempili, and menjalin had 17 to 21% damaged fibers or about double in the 3rd beating. Although it is uncertain, it is assumed that the low specific gravity value in sempori caused less resistance to mechanical action and generated more damaged fibers, whereas the higher thickness of the wall of kempili would cause the least damage in the 3rd beating.

Table 4. Damage proportion (%) in fiber of kraft pulp at three-step beating from five species grown in natural forest

No	Species	1st		2nd		3rd	
		Intact	Damage	Intact	Damage	Intact	Damage
1	Sempori	75.67	24.33	69.50	30.50	51.06	53.92
2	Cempaka	92.92	7.08	83.53	16.47	74.84	25.16
3	Mentawa	87.65	12.35	81.79	18.21	80.48	19.52
4	Kempili	91.39	8.61	89.12	10.88	82.40	17.60
5	Menjalin	89.07	10.93	85.35	14.65	78.81	21.19

After evaluating the damage, the dimensional changes on the fibers were also measured (Fig. 2). The length and thickness of the fibers decreased, while their diameter or width and lumen showed an increasing trend with an increasing number of beating (Fig. 2). Therefore, at the 3rd beating, fiber diameter, and lumen reached maximum values, while fiber length and wall thickness obtained minimum values. However, slight changes were observed in kempili and sempori for fiber diameter as well as cempaka and menjalin for fiber wall thickness at each stage of beating. Considerable decrease in fiber length was observed in maceration sample to 1st beating for mentawa and menjalin samples. A remarkable reduction in fiber wall thickness was observed only in kempili fibers. The calculation of fiber dimension reduction or addition at the 3rd beating based on the values of the control (macerated samples) is presented in Table 5. The highest reduction in fiber length as well as addition in fiber diameter and fiber lumen diameter was observed in menjalin. In contrast, the highest reduction in fiber wall thickness (29.22%) was found in kempili. In this experiment, menjalin had the highest value of specific gravity while kempili had the highest value of fiber diameter, fiber lumen diameter, and fiber wall thickness. Therefore, this indicates the relationship between specific gravity or fiber wall thickness and the dimensional changes after beating.

The effects of beating on the derived values were generally as expected, with the gradual slenderness ratio, Runkel ratio, coefficient rigidity values decreasing (Figure 2a) and flexibility coefficient values increasing (Fig. 1). The magnitudes of the dimensional changes were measured at the 3rd beating based on the values of the maceration samples (Table 5). Observations showed that mentawa and sempori pulps had greater decreases in coefficient rigidity (22~23%), while sempori pulp in Runkel ratio (24.95%) and slenderness ratio (41.43%). The highest increasing level in flexibility coefficient (9.90%) was observed in the kempili wood. There is no clear pattern of dimensional change levels with the specific gravity of these species.

Table 5. Dimensional changes (%) of kraft pulp at third step beating from five species grown in natural forest

No	Fibre properties	Sempori	Cempaka	Mentawa	Kempili	Menjalin
1	Fibre length	-24.00	-12.68	-19.2	-19.13	-29.23
2	Fibre diameter	+3.43	+6.06	+16.09	+0.63	+17.47
3	Fibre lumen diameter	+18.67	+16.47	+3.46	+21.79	+31.75
4	Fibre wall thickness	-21.06	-5.82	-10.70	-29.22	-5.26
5	Runkel ratio	-24.95	-18.53	-21.40	-15.50	-9.53
6	Slenderness ratio	-41.43	-17.64	-30.41	-19.66	-39.75
7	Flexibility coefficient	+7.58	+8.80	+5.27	+9.90	+5.37
8	Coefficient of rigidity	-22.68	-11.15	-22.92	-14.42	-19.17

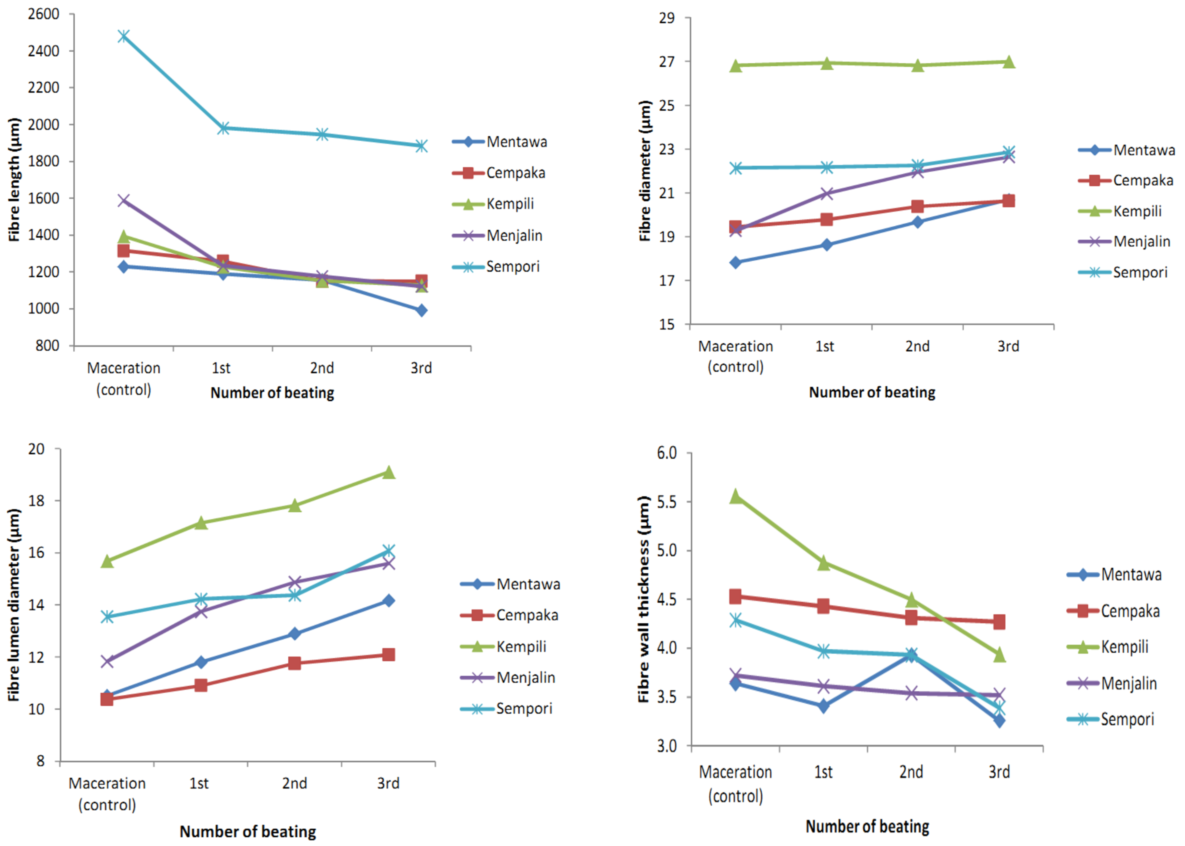

Figure 2. Fiber dimension of kraft pulp at three-step beating from five species grown in natural forest

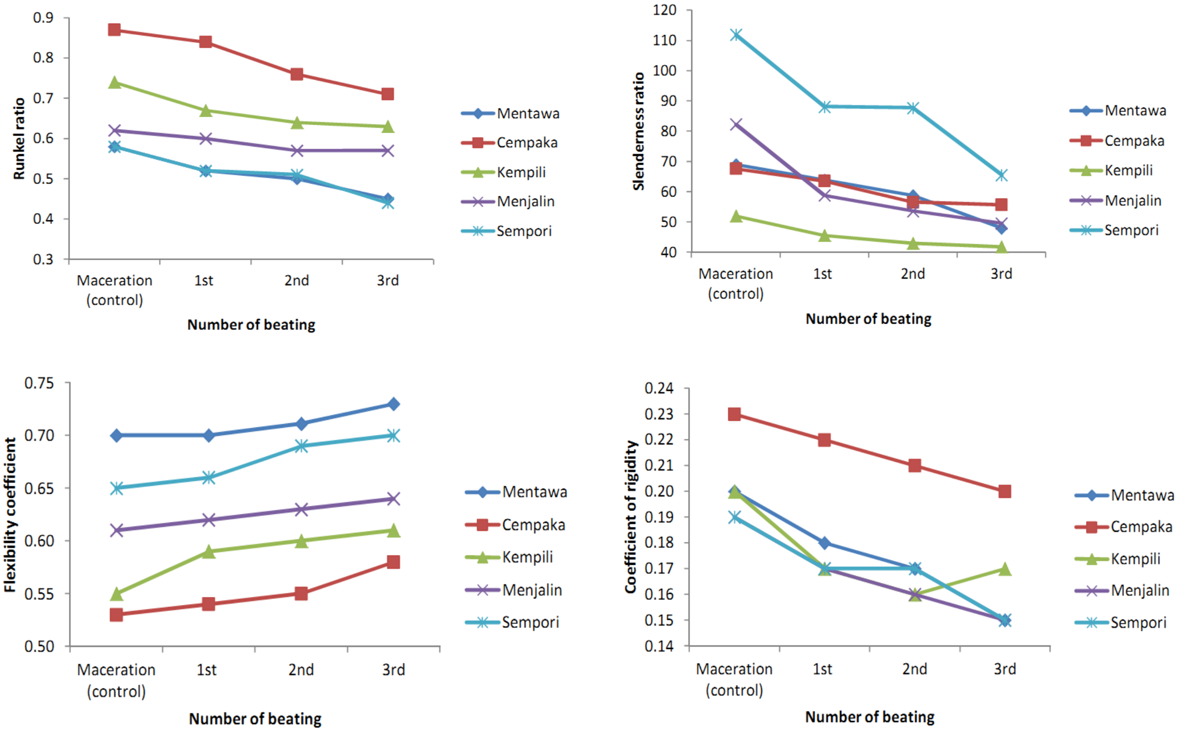

Figure 3. Derived values of kraft pulp at three-step beating from five species grown in natural forest

Paper Mechanical Properties

The differences in paper strength among the five hardwood pulps were as expected. The initial quality and strength of the fibers, as well as the bonding between them, affect the strength properties of the paper. Paper made from unbeaten fibers has low bulk, low strength, and a rough surface, which are commonly undesirable for various end products. The comparison among the species in mechanical properties of the sheet at the 1st beating is presented in Table 1. The maximum level in the tensile index were observed in cempaka and kempili, while the maximum level in the burst index was observed in cempaka and mentawa. Sempori had the highest value in tear index. Tensile index and tear index are two important indicators of physical properties of paper, and they depend on the fiber strength, fiber binding force, and the fiber length (Zhang and Xia, 2014; Seth 1990). The relatively high values in fiber length and slenderness ratio in simpori may explain the high value of tear strength. Cempaka and kempili had the highest fiber wall thickness value, which was related to their high level of tear index. In addition, cempaka and mentawa had the lowest values in fiber diameter in the first beating process, which might relate to their high value in the burst index.

Based on the Indonesian National Standard (SNI) for leaf (hardwood) bleached kraft pulp in terms of tear index, no species met the standard. Sempori and menjalin also failed to meet the standards for tensile and burst indices. This suggests that cempaka, mentawa, and kempili have more opportunities to be developed as raw materials for pulp and paper. However, this experiment was based on only one pulping condition. Since pulp and paper quality also depends on process factors, further work should be conducted for process optimization for each species.

Table 6. Paper mechanical properties at first beating step from five species grown in natural forest

No	Mechanical Properties	Tensile index (Nm/g)	Burst index (KPa m^2/g)	Tear index (mNm^2/g)	SG
1	Sempori	41.92	1.83	5.02	0.58
2	Cempaka	60.94	3.49	3.75	0.60
3	Mentawa	57.51	3.48	4.30	0.66
4	Kempili	60.06	2.65	4.49	0.66
5	Menjalin	42.12	2.27	4.21	0.68
6	Leaf bleached kraft pulp (SNI) [a]	45.00	2.50	5.50	

Remark: a = Indonesia National Standard (SNI 6107, 2009), SG = specific gravity

Effect of Re-beating on Mechanical Properties

Beating increased the hydrogen bonding capacity of the fibers, and consequently the strength of the fiber network but decreased the strength of individual fibers (Tonoli *et al.* 2013). The bonding between fibers is increased because of an increase in fiber collapsibility, specific surface area, and flexibility. Beating process conditions must be selected to maximize the desired effects and minimize the undesired effects (Hietanen and Ebeling 1990). Mechanical properties (i.e. tensile index, burst index, and tear index) decreased with the increased beating number (Table 7). The strength values were highest for the paper sheet subjected to the 1st beating (530~660 mL CSF) compared to the lowest for the 3rd beating (220~230 mL CSF). The degree of reduction among the species was almost similar for tear index, but it varied for tensile and burst indices. The magnitude of reduction (percentage) at the 2nd and 3rd beatings based on the strength values at the 1st beating is summarized in Table 6. In terms of tensile index, menjalin wood showed the lowest level of reduction, indicating it has more resistant fibers to mechanical actions. The different species were observed to give the lowest reduction at the 2nd and 3rd beatings for burst and tear indices.

Table 7. Decrease of paper mechanical properties (%) at second and third beating steps

No	Species	Tensile index		Burst index		Tear index	
		2nd	3rd	2nd	3rd	2nd	3rd
1	Sempori	35.06	47.11	26.28	28.85	41.65	71.05
2	Cempaka	33.91	43.61	49.64	54.71	31.46	49.44
3	Mentawa	23.83	52.49	24,21	47,04	27.72	61.82
4	Kempili	42.71	51.94	35.01	44.13	41.72	62.87
5	Menjalin	10.66	16.36	18.56	37.69	43.06	62.34

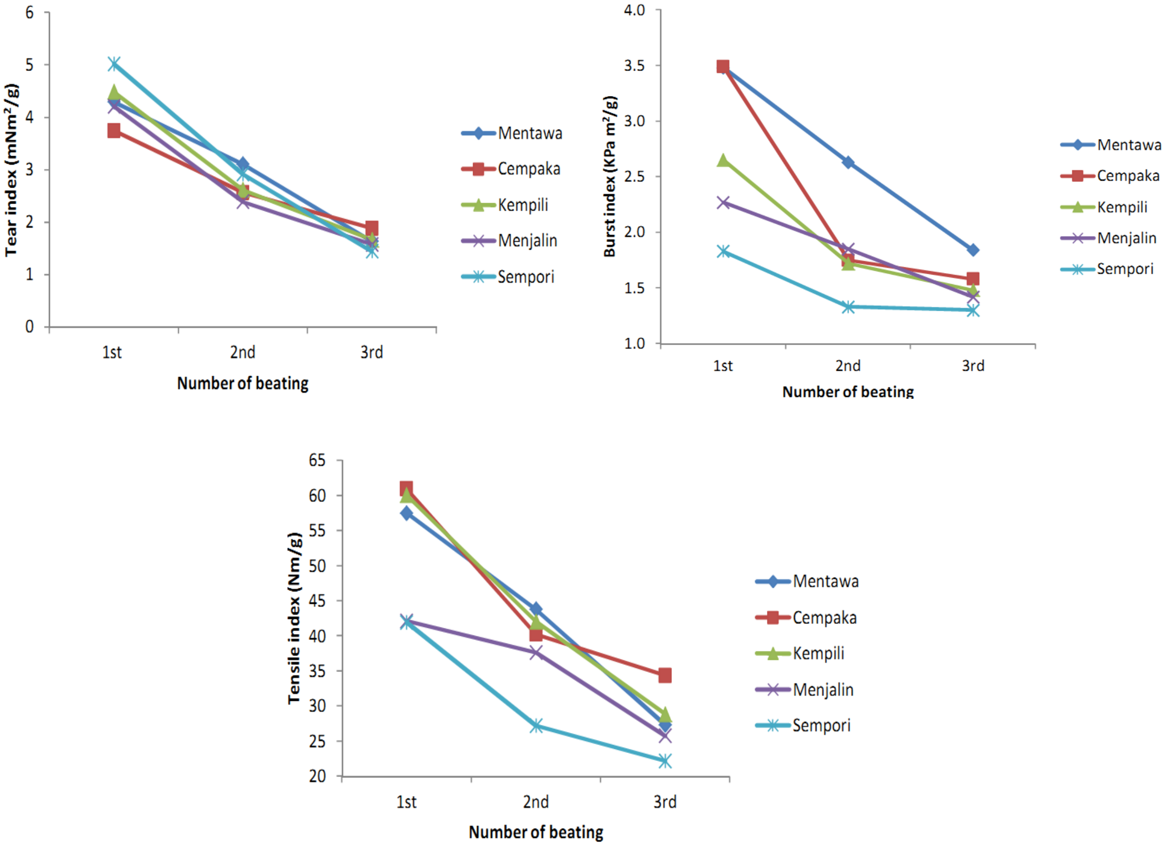

Figure 4. Strength properties of paper at three-step beating

The main factors affecting the tensile index of paper were the strength of the fiber combination and the average fiber length (Gao *et al.* 2012). The change in tear index should also be attributed to the derived values of the fibers. When the beating number was only 1st, the strength of a single fiber was good, while there was little friction between fibers, resulting in a lower tear index. As the beating number increased, the slenderness ratio (the ratio of fiber length to fiber width) decreased, while their strength and the combination and friction between fibers increased. Technically, the higher values of flexibility coefficient along with the lower values of Runkel ratio and rigidity coefficient after beating would increase the paper strength. On the contrary, the decreasing value of the slenderness ratio after beating would decrease the paper strength. Thus, it is assumed that the changes in the slenderness ratio that was reduced had more considerable effect as compared to the other derived values.

As the number of beating increased, the beating degree of the pulp continuously decreased, while the average fiber length became shorter and shorter (Fig. 2). Fiber cutting or fiber length reduction could negatively affect the paper strength values, such as tensile index and tearing resistance (Rosli *et al.* 2011). Under the influence of the fiber bonding force and average fiber length, the tear index decreased after the point of maximum value, which indirectly indicated that excessive fiber cutting would decrease the tear strength of the paper. When the average fiber length decreased, it became the main factor affecting the burst index and caused its decrease (Gao *et al.* 2012). Burst index, which analyses the resistance to a uniformly distributed pressure under test conditions, is positively influenced by the fibrillation promoted by the refining process and negatively influenced by the shortening of the fiber length (Mutje'a *et al*. 2005).

Beating or refining both causes damage to the fibers (fiber shortening, fiber damage) and increases the bonding strength (Molin *et al.* 2004). Optimal beating is therefore an optimization of parameters to obtain maximum bonding strength without an excessive decrease in fiber strength and more energy savings. It is expected that wood with high specific gravity would need more intense beating. In terms of production, the 1st beating produced the best strength of paper sheet and consumed less energy for all species. However, additional treatments are necessary for recycled paper purposes, which require multiple beatings due to considerable strength reductions. In previous work, it was found that the maximum level of tensile and burst indices was observed in the 200~300 mL CSF range, while the tear index was observed in the 400~600mL CSF range for *Acacia mangium, Acacia auriculformis*, and Acacia hybrid pulps (Yamada *et al.* 1992). In addition, the paper strength is set at 300 ml CSF in SNI. It is uncertain whether the range of 530~660 mL CSF is optimal for higher wood density such as these observed species. Therefore, future studies should employ a wider range of wood density to confirm this hypothesis.

Conclusions

Specific gravity, fiber morphology, and pulp and paper properties were determined for five lesser-known species - mentawa, cempaka, kempili, menjalin, and sempori. These species showed high specific gravity values (0.58~0.68) for pulp and paper purpose and potential values for fiber length (1239~2479 μm) and derived fiber values, Runkel ratio of 0.58~0.87 and slenderness ratio of 52~112. The pulp screened yield and kappa number values were 24~44 and 17.2~19.4, respectively. After three phases of beating, it showed that damaged fibers, as well as fiber length, fiber wall thickness, Runkel ratio, slenderness ratio, and coefficient rigidity, decreased with increasing beating number. Regarding strength properties, cempaka, kempili, and mentawa woods were characterized as sheets with higher tensile and burst indices. The strength values were highest for the paper sheet subjected to the 1st beating (530~660 mL CSF) compared to the lowest at the 3rd beating (220~230 mL CSF). It is noticed that decreasing the slenderness ratio level had a more significant effect compared to other derived values.

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WOOD RESEARCH Journal

Journal of Indonesian Wood Research Society

Annals of the Wood Research Journal

Wood Research Journal is the official journal of the Indonesian Wood Research Society. This journal is an international medium in exchanging, sharing and discussing the science and technology of wood.

Aims and Scope

The journal publishes original manuscripts of basic and applied research of wood science and technology related to Anatomy, Properties, Quality Enhancement, Machining, Engineering and Constructions, Panel and Composites, Entomology and Preservation, Chemistry, Non Wood Forest Products, Pulp and Papers, Biomass Energy, and Biotechnology. Besides that, this journal also publishes review manuscripts which topics are decided by the Editors.

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Publication Frequency

Journal is published in one volume of two issues per year (April and October).

Peer Review Policy

WRJ reviewing policies are: Every submitted paper will be reviewed by at least two peer reviewers. Reviewing process will consider novelty, objectivity, method, scientific impact, conclusion and references.

General Remarks

Manuscripts will be accepted for publications are those discussing and containing results of research on wood science and technology, and reviews on specific topics, which are decided by the Editors and have not been published elsewhere. Authors are requested to correct the manuscripts accepted for publications as suggested by the Reviewers. Editors could change positions of Figures and Tables.

Manuscripts Preparations

1. Manuscripts must be in English, typewritten using Word, Arial Narrow, single space, 3 cm of left and right margin and 2.5 cm of top and bottom margin of a Letter paper size. Title is printed with a font size of 14 pt, Authors are of 12 pt, and Text is of 10 pt.
2. Manuscripts should be checked for spelling and grammar by a native speaker.
3. Manuscripts compositions:

3.1. Title
3.2. Complete name of Authors
3.3. Abstract
3.4. Key words
3.5. Texts:
    Introduction
    Materials and Methods Results and Discussion
    Conclusions (and Suggestions) References
    Name and complete address of Authors
    Appendix
    4.Other rules:
4.1. Names of wood are followed by Botanical Name.
4.2. Values between are written using this symbol (⍰), e.g. 3.75 ⍰ 8.92%.
4.3. Editors could modify Figures without changing their substantial meaning.
4.4. References are arranged from A to Z.
4.5. References in text are written as this example: (Palomar *et al.* 1990; Arancon 1997).
4.6. Examples of writing of References: Altschul, S.F.; T.L. Madden; A.A. Schäffer; J. Zhang; Z. Zhang; W. Miller; D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs. Nucleic Acids Res. 25: 3389-3402.

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Example of Table and Figure

Table 1. Effects of temperature on *in vitro* growth of seedlings.

Temp. (°C)	Shoot length (mm)	Number of leaf	Fresh weight (g)
25	59.2 ± 10.6^{c}	4.5 ± 0.8^{a}	0.29 ± 0.13^{a}
27	88.5 ± 9.3^{a}	4.8 ± 0.9^{a}	0.40 ± 0.12^{a}
29	75.0 ± 11.1^{b}	3.8 ± 0.6^{a}	0.30 ± 0.07^{a}

Note: Values (average ± standard deviation) with different letters are statistically significant according to Tukey's multiple comparison test. Data were recorded after 4 weeks of culture. MS medium was used as a basal medium without any PGRs. Number of sample = 10.

Source: Chujo *et al* 2010.

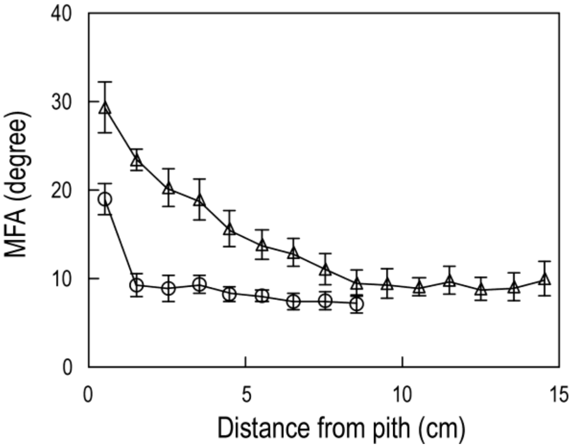

Figure 3. Radial variation of microfibril angle of the S2 layer in tracheid. Open circle, *Agathis* sp.; open triangle, *Pinus insularis*; Bars indicate the standard deviation. (Source: Ishiguri *et al* 2010)

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