

ISSN 1819-1894

Asian Journal of
Agricultural
Research

Improved Physical and Chemical Properties of Rubber Wood (*Hevea brasiliensis*) Fiber by Laccase

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ABSTRACT

Cellulose and lignin are the two most abundant polymers found in plant cells. Cellulose microfibrils provide mechanical properties to woody cell whereas, hemicelluloses and lignin act as glue in between the crystalline cellulose. Cellulose crystallinity directly affects the physico-chemical behavior of the individual fiber such as modulus, hardness, stiffness, tensile and swelling-shrinkage properties and ultimately to its product. This study aims to improve the physico-chemical properties of rubber wood fiber by laccase hydrolysis in order to improve the crystalline structure. Two different times of reactions i.e., 60 and 120 min were applied at constant temperature 25°C, enzyme concentration 7 U g⁻¹ and pH 5. After the pulp treatment, fibers were sieved out and transferred in to autoclave at 121°C for 15 min to stop further enzyme reaction. Treated fiber was dried in electric oven drier at 80-90°C for 24 h to dry up the fiber until zero moisture content. Crystallinity index of the fiber was measured by X-ray diffraction method and it was observed maximum up to 14% higher compared to untreated fiber. The treated fiber was further analyzed for elemental composition, FE-SEM and TGA and compared with untreated fiber in order to evaluate its properties. Fiber treated with enzyme exhibited superiority in fiber surface structure and thermal degradation over untreated fibers. A fiber with improved mechanical strength and crystalline structure can be utilized for various purposes to produce high quality product.

Key words: Crystallinity index, laccase, elemental analysis, FE-Scanning electron microscope

INTRODUCTION

Wood cell walls are composed of cellulose, hemicelluloses and lignin (Stefanidis *et al.*, 2014). The mechanical property of wood fibers is mainly determined by cellulose and hemicelluloses, whereas lignin acts as an adhesive between the fibers (Winandy and Rowell, 2005; Gea *et al.*, 2011). Cellulose is a long polymer chain of linear crystalline interrupted with regular amorphous region, primarily responsible for strength in the wood fiber (Stefanidis *et al.*, 2014; Nazir *et al.*, 2013). Hemicelluloses are amorphous, highly branched polymer compared to cellulose (Zhou *et al.*, 2009), as a linkage between cellulose and lignin. Lignin, on the other hand, is an amorphous phenolic compound that functions as a cementing material and stiffening agent for the cellulose molecules within the fiber cell wall (El Mansouri *et al.*, 2007). Most of the lignin is found inside the cell wall

as an intra-cellular lignin (70%), the rest are found in the middle lamella as extracellular lignin (Tuor *et al.*, 1995; Kunamneni *et al.*, 2007).

Laccase is a well-studied oxido-reductase enzyme, it takes part in polymerization as well as depolymerization of lignin compound through free radical reaction (Nasir *et al.*, 2013). Since, laccase enzymes are too large to penetrate into fibers (55-80 kDa), it modifies the amorphous extracellular component only (Gochev and Krastanov, 2007). Hence, due to a size exclusion reasons, laccase treatment is only a surface modification and it alters extracellular lignin without affecting the cellulose fiber. Although, several researchers have studied the enzymatic hydrolysis of lingo-cellulosic fiber but the changes in physical and mechanical properties of fiber is still not fully understood (Yaropolov *et al.*, 1994; Kharazipour *et al.*, 1997). Thus, this study deals the pretreatment reaction of laccase at two different times, on natural fiber and the result was compared with untreated fiber. Such improved fibers can be used in many industrial applications like fiber board, pulp and paper industries etc (Virk *et al.*, 2012; Nasir *et al.*, 2013; Wu *et al.*, 2011). This work aims to improve the physico-chemical properties of rubber wood fiber by laccase hydrolysis in order to improve the crystalline structure.

MATERIALS AND METHOD

Material: A thermo-mechanically processed pulp of rubber wood fibers was supplied by Robin Resources (Malaysia) Sdn. Bhd. The pulp was then air dried to moisture content of 15-20% before applying any treatment. Laccase enzyme (Novo WA 20040) was supplied by Novozyme, Malaysia.

Enzymatic pulp treatment: About 25 g of oven dried wood fibers of was suspended in 475 g of deionized water to make a solution of 5.0% consistency (mass pulp/mass suspension) in a 2 L Erlenmeyer flasks. A buffer solution of sodium acetate and acetic acid was used to maintain the pH. Two different reaction time (60 and 120 min) were applied at constant temperature of 25°C, enzyme concentration of 7 U g⁻¹ and pH 5 (Nasir *et al.*, 2014). The two treatment were named as FT1 (Fiber treated) and FT2 when treated at 60 and 120 min, respectively, whereas untreated was named as UT in later part of result and discussion. After each pulp treatment, fibers were sieved out immediately from the solution and transferred in to autoclave at 121°C for 15 min to stop further enzyme reaction. Treated fiber was dried in electric oven drier at 80-90°C for 24 h to dry up the fiber until zero moisture content.

Elemental analysis: Elemental analysis of treated and untreated fiber was performed in Central Laboratory, University Malaysia Pahang. Samples were analyzed on Varo Macro Cube, S/N-2012/1005, by simple combustion process.

Crystallinity: X-ray measurements were conducted on a Rigaku MiniFlex II, bench top X-ray Diffractometer (XRD) analyzers. The fiber specimens were pressed at 10 t hydraulic pressure into a circular shape of a tablet with 14 mm diameter and 0.5 mm of thickness. The X-ray diffractometer was operated at a voltage of 30 kV with a current density of 15 mA. The scanning range was from 2 θ = 10-50° at a scan speed of 0.015° sec⁻¹. The data was collected using a fixed time mode with angular intervals of 0.015°. The method adopted was based on Kim and Holtzapple (2005). Crystallinity Index (CrI) was calculated from Eq. 1:

$$\text{Crystallinity index (CrI)} = \frac{I_{002} - I_{\text{am}}}{I_{002}} \times 100 \quad (1)$$

where, I_{002} is the intensity of the diffraction from the 002 plane $2\theta = 22.0-23.0$ and I_{am} is the intensity of the background scatter measured at $2\theta = 16.0-18.0$.

RESULTS AND DISCUSSION

Fiber crystallinity index: An initial experiment was carried out to understand the real effect of laccase treatment on rubber wood fiber. Figure 1 exhibited, the untreated fibers having the least crystallinity index of 65.5% whereas, it was improved in treated fibers to 76.4 and 72.87% in FT1 and FT2, respectively. The X-ray-based measurement of Crystallinity Index (CrI) indicates that laccase treatment increased the crystallinity of rubber wood and maximum was observed at 1 h of treatment time. However, the CrI started to reduce when the reaction was prolonged for 2 h. The change in crystallinity index was due to the removal of extracellular lignin from the fiber surface that ultimately improves the crystallinity (Lionetto *et al.*, 2012; Wu *et al.*, 2011) but in the next 3 h fiber showed a decreasing trend. It was expected that a prolonged treatment, lignin started to precipitate back on the fiber surface that results in the fall of crystallinity which is supported by the FE-SEM result (Kumar *et al.*, 2009).

Elemental composition of treated fiber: Elemental composition (CHNS) of untreated fiber and treated fibers were analyzed. Table 1 showed the mean of element percent of two tests' result. The treated and untreated fibers exhibit a little difference. An untreated fiber displayed the lowest carbon percentage (C%) and highest hydrogen (H%) whereas, it was reverse in treated fibers. Since two processes, lignin breakdown and deposition taking place together, its elemental composition does not give a clear difference thus it was assumed to be insignificant.

Microstructure analysis: Figure 2 depicted the FE-SEM picture of treated and untreated fiber at 5000x magnification. From the close view of micrograph, it was obvious that an enzyme treatment brought a distinguish change in the fiber surface. An Untreated Fiber (UT) had uneven furrowed surface whereas, a treated fibers have a very smooth surface. The uneven surface of wood fiber could be due to extra-cellular lignin found naturally on fiber surface which was either removed in lignification process or become even surface due to deposition. Furthermore, FT2 fiber exhibits slightly thicker layer of deposition as compared to the FT1 treated fiber. Although, laccase act

Table 1: Elemental composition of treated and untreated fiber

Samples	C (%)	H (%)	N (%)
UT	43.636	6.392	0.398
FT1	43.7295	6.3885	0.715
FT2	45.4985	6.152	0.823

UT: Untreated fiber, FT: Fiber treated, C: Carbon, H: Hydrogen, N: Nitrogen

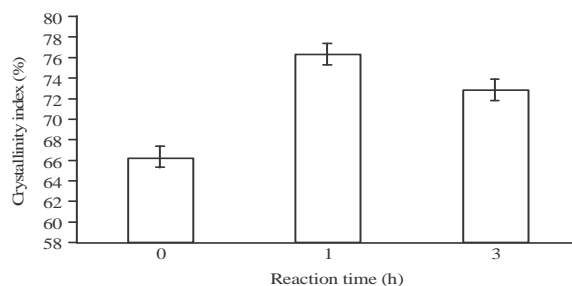


Fig. 1: Fiber crystallinity index of treated at enzyme concentration of 7 U g^{-1} and pH 5

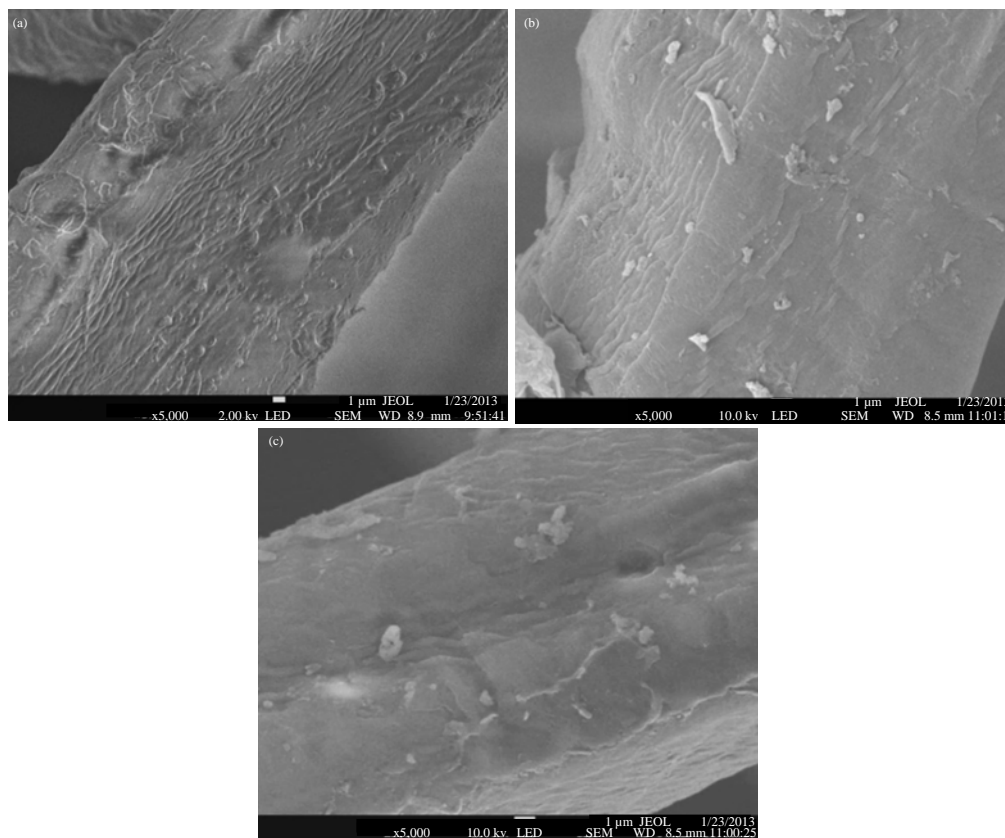


Fig. 2(a-c): FE-SEM of (a) UT, (b) FT1 and (c) FT2 treated fibers at different reaction condition at 5000x magnification

specifically on lignin, the deposition composition should be studied thoroughly. Thus, it was concluded that the smoothness of the fiber surface could be due to the removal of loosely bonded lignin and then precipitation of the hydrolyzed lignin, as a smooth layer on the fiber surface which is similar to the finding of Kumar *et al.* (2009).

Thermogravimetric analysis (TGA) analysis of treated fiber: Thermal stability of treated and untreated fibers was analyzed on TGA. Thermal degradation of wood fibers occurs in two stages, it initiates from the amorphous cellulose at around 300°C followed by the crystalline cellulose degradation at a higher temperature around 350°C (Quintana *et al.*, 2015). From the Table 2, it was observed that initial degradation of untreated fiber (UT) was started at temperature 331°C whereas, this temperature was found higher for treated fibers (FT1 and FT2). Final degradation temperature of treated fiber was also increased from 449°C of untreated fiber to 491°C of treated fiber. Among the treated fibers, FT1 exhibits higher degradation temperature as compared to FT2 due to its higher CrI. Transition temperature (T_g) was calculated as a mid-point of onset and end point from TGA curve and summarized in Table 2. It was observed that T_g value of treated fiber was higher and increases with the increase in crystallinity of the fiber.

Figure 3 shows the DTA curves were all wood fibers (either treated or untreated fibers) exhibited two exothermic peaks overlapping with regions of weight loss (Jawaid and Khalil, 2011).

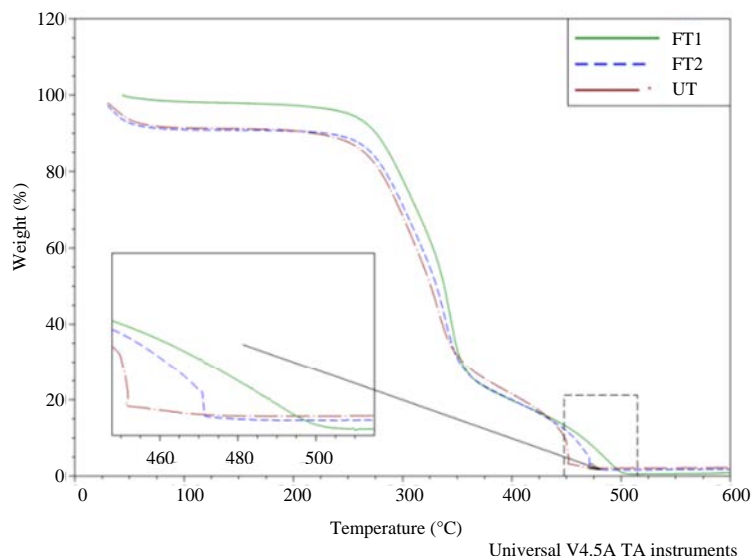


Fig. 3: Thermogravimetric analysis showing a two-step decomposition of fiber

Table 2: Transition temperature value and weight loss obtained from thermogravimetric analysis graph

Samples	Onset (°C)	Mid-point (Tg)	End (°C)	Weight (mg)	Weight (%)
FT1	308.86	331.485	354.11	2.023	75.75
FT2	298.09	324.64	351.19	4.026	68.96
UT	284.56	314.255	343.95	5.452	65.85

Tg: Transition temperature, FT, Fiber treated, UT: Untreated fiber

The untreated rubber wood fiber started to degrade at 331°C were as this temperature was increased to 340 and 344°C for FT2 and FT1 samples, respectively. Therefore, it was concluded that a treated fiber were having a higher decomposition temperature as compared to untreated fiber (Li and Pickering, 2008).

CONCLUSION

Crystallinity index was observed maximum up to 76%, at 1 h enzyme reaction condition then after it decreased considerably and remained constant for a wide range of around 71-72%. Fiber treated with enzyme exhibited superiority in fiber surface structure and thermal degradation over other treated and untreated fibers. Thus, to obtain a highly crystalline and mechanically strong fiber, a thorough study of various reaction parameters such as time, concentration and temperature of the reaction is recommended. A fiber with improved mechanical strength and crystalline structure can be utilized for various purposes to produce high quality product.

ACKNOWLEDGMENTS

The authors acknowledged Universiti Sains Malaysia for Post-doctoral fellowship to Dr. Mohammad Nasir.

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