

Laccase-Catalyzed Fiber Functionalization with Different Phenolic Compounds for Enhancing Pulp Strength

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Chemo-enzymatic functionalization offers an innovative approach to produce paper and board products with enhanced performance. Unbleached softwood kraft pulps were functionalized by laccase with methyl syringate (MS), p-hydroxybenzoic acid (HBA), gallic acid (GA), and syringaldehyde (SyA). The wet strength of fibers treated with MS and SyA increased by 57.9% and 31.9%, respectively. The dry strength of fibers treated with HBA, GA, and SyA increased from about 64 N·m/g to 68 N·m/g. The opacity of MS-treated fibers was the highest, and the surface lignin coverage increased. The kappa number and surface lignin of HBA-treated fibers changed little; however, the total carboxyl group significantly increased. The participation of phenolic compounds enhanced the reactivity of fibers to laccase in varying degree. However, the reactivity of phenols to laccase did not show a direct relation to the paper strength. All treatments with phenols decreased the brightness and the curl index of fibers. The syringyl-type phenols with hydrophobic groups (OCH₃) were shown to be effective for improving the pulp wet strength. The compounds with carboxyl groups enhanced the pulp dry-strength. The observed pulp strength improvement could be attributed to the formation of covalent bonding via radical coupling, the attachment of the functional group, increased bonding area, and fiber entanglement.

Keywords: Laccase; Fiber; Functionalization; Phenolic compound; Pulp strength

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INTRODUCTION

Enzymatic functionalization is an attractive tool that provides a reactive interface for processing lignocellulosic materials. Laccases are glycoproteins that catalyze the oxidation of phenols and aromatic or aliphatic amines to the corresponding reactive radical with concomitant reduction of molecular oxygen to water (Durán *et al.* 2002). Laccase-catalyzed functionalization of pulp is based on the activation of the fiber surface, followed by the attachment of novel functional groups to fiber by radical coupling (Suurnäkki *et al.* 2010). The attached group or molecule will introduce new properties to fibers and strength to the fiber material. Compared with the conventional chemical methods, the laccase-catalyzed grafting method provides an eco-friendly process and “clean” technology.

The potential of laccase for functionalizing lignocellulosic fibers with various compounds has been studied. The laccase-assisted biografting of phenolic compounds for improving strength properties of kraft paper made from high-kappa pulps was discussed in previously papers by Chandra and co-workers (Chandra and Ragauskas 2002a,b; Chandra *et al.* 2004a,b). Laccase treatment combined with tannic acid enhances the adhesion of fibers in the environmentally friendly production of fiberboard (Widsten *et al.* 2009). The internal bond of particle boards is significantly enhanced by laccase-catalyzed

functionalization with 4-hydroxy-3-methoxybenzylurea (Fackler *et al.* 2008). Recently, some researchers have focused on using laccase and some phenolic compounds for conferring antibacterial properties to lignocellulosic materials (Fillat *et al.* 2012; Kudanga *et al.* 2010a).

A variety of amino acids have been used to functionalize pulps for improving the paper strength (Witayakran and Ragauskas 2009; Chen *et al.* 2010). The grafting of fluorophenols and long chain alkylamines on wood fibers can increase the hydrophobicity of the wood fiber surface (Kudanga *et al.* 2010c). The strongest internal sizing effect resulted when the kraft pulp was treated by laccase with hydrophobic compounds (Garcia-Ubasart *et al.* 2011).

The fiber modification mechanisms by laccase with different compounds are very complex. As is well known, phenoxy radicals are formed in the lignin matrix in the laccase-catalyzed oxidation of wood fibers (Felby *et al.* 1997). Activation of fibers is the first step in fiber functionalization aiming at an introduction of desired properties to fiber (Grönqvist *et al.* 2006). A variety of factors affect activation of fibers, including pulp type, laccase dosage, treatment time, oxygen addition, and type of functional compound (Suurnäkki *et al.* 2010).

The further reactions after fiber activation may be polymerization, depolymerization, copolymerization, or grafting. The functional compounds with different chemical structure may confer upon fibers some new properties (Aracri *et al.* 2010). The laccase treatment combined with ferulic acid can increase the kappa number and the surface anionic charge of unbleached sisal pulp (Aracri *et al.* 2011). Also, pulp refining prior to enzymatic treatment can further improve strength-related properties (particularly wet tensile strength) of the resulting paper. Recently, the laccase treatment combined with five phenolic compounds was first applied to kenaf pulp (Andreu and Vidal 2011). All the phenolic compounds increased kappa number, decreased brightness, and changed optical properties of the pulp after the laccase stage. However, the syringaldehyde and acetosyringone provided the greatest delignification and bleaching effects after the alkaline and H₂O₂ stage.

There have been several studies on the use of several lignin-derived phenols as laccase mediators for aiding pulp delignification in a bleaching sequence (Fillat *et al.* 2010, Nousiainen *et al.* 2009; Camarero *et al.* 2007). However, some plant phenols such as ferulic acid and coniferyl aldehyde proved ineffective for bleaching of sisal pulp and exhibit a tendency to couple onto fibers (Aracri *et al.* 2009). Certain phenolic compounds such as sinapinaldehyde and vanillin can increase laccase stability and act as enhancers in laccase-catalyzed reactions (Díaz-González *et al.* 2011).

In summary, the chemical structure of phenolic compounds can have important influence on the fiber properties when the pulp is functionalized by laccase. In this work, some phenolic compounds with different functional groups, such as methoxyl, phenolic hydroxyl, and carboxyl groups were used to functionalize the unbleached softwood kraft pulp by laccase catalysis for enhancing the paper strength, including dry and wet strength. The actions of different phenolic compounds in functional reactions were studied. The primary objective of the present work is to give an insight on the bonding mechanisms behind the laccase-induced fiber functionalization with different phenolic compounds and elucidate the correlations between the structure characteristics of functional compounds and the dry/wet tensile strength of pulp.

EXPERIMENTAL

Pulp, Laccase, and Phenolic Compounds

The unbleached softwood kraft pulp was prepared from a commercial pulp board with a kappa number of 27 (Chenming Paper Group, Shandong, China). Prior to the laccase treatments, pulp samples were refined with a PFI mill to 45 °SR. The liquid laccase, Novozym 51003, was supplied by Novozymes A/S (Beijing, China). The laccase activity (1000 U/g) given by the producer was expressed in units (U) with 1 U defined as the amount of enzyme that oxidizes 1 mmol of syringaldazine per minute in a Na-acetate buffer (pH 4.5) at 55 °C (Xu *et al.* 2011). The methyl syringate (MS), *p*-hydroxybenzoic acid (HBA), gallic acid (GA), and syringaldehyde (SyA) were analytical grade.

Pulp Treatments

Laccase (10 U/g dry pulp) and phenolic compounds (0.5%; w/w) were added to a 5% consistency sample at pH 4.5, buffered to such pH by addition of 0.1 M acetate buffer. The resulting slurry was stirred in a water bath at 45 °C under O₂ atmosphere bubbling for 2 h. The control sample was treated under the same conditions but without addition of enzyme and phenolic compounds. Five handsheets were made from each pulp sample and 12 test strips were used for wet tensile strength testing.

Handsheet Testing and Analysis of Pulp Properties

After the enzymatic treatment of pulp, handsheets with a grammage of 60 g/m² were immediately made using the TMI TAPPI standard sheet apparatus, according to the TAPPI T205 method. Six handsheets were made from each pulp sample, and 18 test strips were used for dry and wet tensile strength testing according to TAPPI Standard T494. The tensile strength was tested with a Tensile Strength Tester from Lorentzen and Wettre. The wet-tensile index was measured from sample strips soaked in deionized water for 1 h. The optical properties were determined on a Datacolor Elrepho 2000 Tester according to TAPPI T452.

The kappa number of fibers was measured according to TAPPI T236. The total acid group content was determined using conductivity titration method, according to TAPPI T237. Each pulp was washed with deionized water up to a neutral pH before testing the kappa number and carboxyl group of fibers. Both values are means of three replicates. The data from fiber quality analysis (FQA) are the average values of five replicates.

Oxygen Consumption Measurement

The oxygen consumption measurements were performed with a dissolved oxygen meter equipped with an O₂ probe at room temperature. Dry kraft pulp (2.5 grams) was dispersed in 0.05 M sodium acetate buffer (pH 4.5) in a 250 mL conical flask without headspace. The mixture was saturated with O₂ for 5 minutes at room temperature followed by addition of 0.5% phenolic compounds. The mixture was magnetically stirred at 200 rpm. The probe was instantly introduced into the flask while a dosage of 32 U/g dry fibers laccase was added to the suspension. The oxygen uptake was monitored during 10 minutes. The laccase treatment of phenolic compounds alone was also performed under similar conditions, but without addition of the fibers.

X-ray Photoelectron Spectroscopy (XPS) Analysis

X-ray photoelectron spectra of the handsheet surface were obtained with a physical Electronics ESCALAB 250 instrument from Thermo Fisher Scientific. The position of detector was at a 90° angle with respect to the sample surface. The analyzed surface area was 0.8 mm², and three different spots were analyzed per sample. A Gaussian curve-fitting program was used to deconvolute C1 carbon (C-C, C-H and C=C functional groups) signal at 285 eV. The chemical shifts relative to C1 used in the deconvolution were 1.7 ± 0.2 eV for C-O (C2), 3.1 ± 0.2 for C=O or O-C-O (C3), and 4.3 ± 0.2 eV for O-C=O (C4) groups (Dorris and Gray 1978).

All handsheet samples for XPS analysis were subjected to Soxhlet extraction with acetone and distilled water in series, 4 h for each solvent. The samples were then placed on a clean glass slide, pressed lightly, and then dried in an oven at 60 °C. The smooth-glass side of the sample was used for the XPS measurement. Surface coverage of lignin was estimated according to the following equation developed by Ström and Carlsson (1992). The XPS measurement was conducted at three locations on each handsheet sample. The given data is the average value of three results. Only representative XPS spectra were supplied.

$$\text{Surface coverage of lignin} = (O/C_{\text{after extraction}} - O/C_{\text{carbohydrates}}) / (O/C_{\text{lignin}} - O/C_{\text{carbohydrates}})$$

Atomic Force Microscopy (AFM) Analysis

The samples for AFM were prepared as follows: about 0.2 mL fiber suspension (0.1 g pulp in 100 mL milli-Q water) was dropped onto a purged silicon wafer to ensure single fiber dispersion on the surface and then dried in a vacuum desiccator for 24 h.

AFM measurements were performed using a NanoScope IIIa Multimode scanning probe microscope (Veeco, Santa Barbara, US). All images were obtained using the tapping mode in air at room temperature. Real time scanning was performed at a scan rate of 1.0 Hz, scan angle 0°, and tapping frequencies ranging from 250 to 300 kHz. AFM scanning was conducted at two to four locations on each fiber characterized. Only representative images of samples are shown in the figures.

RESULTS AND DISCUSSION

Pulp Properties

The pulp properties after laccase (L) treatment with different compounds are shown in Table 1. For the laccase-treated pulp, the dry tensile index changed little, and the wet-tensile index slightly increased compared with the control sample. The COOH group also slightly increased and the brightness increased from 23.12 %ISO to 22.22 %ISO. These results indicated that the lignin appeared to be oxidized by laccase. For the laccase treatment combined with different compounds, the dry tensile strengths of all treated pulp were increased in varying degree. Based on the results in Table 1, the compounds with carboxyl groups (HBA, GA) and SyA were able to confer a higher dry strength. A significant increase in COOH content of pulp was observed in the case of L/HBA, L/SyA, and L/GA treatment, in this order. The increase in COOH groups may result from the lignin oxidation or the graft of the compounds with COOH group (such as HBA and GA) on fibers by laccase catalysis. Interestingly, the difference in the total COOH content did not show in the dry tensile strength of these pulps, as it was almost the same after these

treatments. The L/HBA-treated fibers had the largest COOH content and invariable kappa number (compared with the control). The L/GA-treated fibers had the largest kappa number with a lower COOH content, and it had a lower brightness with higher opacity. It was reported (Chandra *et al.* 2004a,b) that the HBA or GA could be grafted on the high-kappa (91) kraft pulp, and the dosage of HBA or GA was shown to have the greatest influence on the kappa number of resulting pulp. In this work, the kappa number of initial pulp was 27. The kappa number changed little after L/HBA treatment; however, the L/GA-treated pulp had the largest kappa number. This needs to be further studied in future work. The HBA presented the least steric hindrance for penetrating to fiber pores. It cannot be excluded that the HBA was not only grafted on fiber but also degraded the lignin in fibers, and this idea is in accordance with the minor brightness decrease of L/HBA-treated fibers. It has been reported that SyA is a mediator in laccase-catalyzed oxidative delignification (Andreu and Vidal. 2011). In their work, the kappa number of L/SyA-treated pulp slightly increased compared with that of the initial pulp; however, the subsequent alkali treatment resulted in a decrease of kappa number. In this work, the kappa number also increased from 24.5 to 26.2 after L/SyA treatment, and its COOH content significantly increased from 15.73 m mol/kg to 20.48 m mol/kg. It is suggested that the increased COOH group mainly results in the lignin oxidation, and the oxidized lignin became easier to remove in the subsequent alkali stage.

The wet-tensile index of L/MS-treated pulp was highest (increased by 57.9%) and that of the L/SyA-treated sample took second place (increased by 31.9%). The wet tensile strength of handsheets was related to bonds being resistant to water sorption, such as covalent bonds between fibers (Espy 1995). The phenolic hydroxyl groups can be oxidized to phenoxyl radical by laccase (Felby *et al.* 1997). The MS or SyA presents the methoxy groups with hydrophobicity. It was suggested that the MS or SyA was probably grafted on fibers by a radical coupling reaction between the phenol compound and surface lignin. The hydrophobic methoxy group and the formation of covalent bonds due to the radical coupling enhanced the wet strength of pulp.

It is interesting that the MS-treated pulp had a higher wet strength but a lower dry strength than the other treated pulps. It has been reported (Page 1969) that at low levels of bonding in the wet sheet, a minor increase in bonding has a significant impact on tensile strength of the handsheet. However, at higher bonding levels in dried sheets, the effect of bonding on dry tensile strength becomes less pronounced (Chandra *et al.* 2004b). Thus, the increased level of bonding force between MS-treated fibers is not enough to further enhance the dry tensile strength, although the increased level of bonding in MS-treated fibers is higher than that in fibers treated by the other compounds with carboxyl group.

Compared with the control pulp, all phenolic compounds employed in the laccase treatments of pulp resulted in an increased opacity and a loss of brightness to varying degrees. Laccase oxidation of phenolic compounds yields phenoxyl radicals. It was suggested that the coupling reaction of the radicals from phenols or surface lignin resulted in the formation of the chromophoric groups. The copolymerization or polymerization led to the increased opacity.

Oxygen Consumption of Fibers and Phenolic Compounds during Laccase Treatment

The reactivity of fibers and different phenolic compounds towards laccase can be determined by oxygen consumption measurements. In order to explore the role of phenolic compounds in functional reactions, the reaction of laccase with different

compounds in the absence or presence of pulp were determined by monitoring the oxygen uptake in the reaction solution in a closed vessel.

Table 1. Handsheet Strengths and Pulp Properties after Laccase(L) Treatment with Different Compounds

Treated Pulp	Dry-tensile index (N·m/g)	Wet-tensile index (N·m/g)	Opacity (%)	Brightness (%ISO)	Total carboxyl (m mol/kg)	Kappa number
Control	63.95±0.28	2.16±0.14	93.40±0.10	23.12±0.10	15.73±0.26	24.5±0.12
Laccase	64.15±0.30	2.39±0.14	94.02±0.12	22.22±0.10	16.45±0.25	24.7±0.14
L/MS	65.50±0.28	3.41±0.14	96.82±0.12	17.80±0.10	17.52±0.20	25.6±0.14
L/HBA	68.32±0.30	2.28±0.14	94.47±0.10	22.50±0.10	22.48±0.25	25.0±0.10
L/GA	67.60±0.32	2.14±0.14	96.35±0.08	17.88±0.10	19.22±0.25	27.8±0.10
L/SyA	68.18±0.30	2.85±0.12	95.25±0.12	20.20±0.10	20.48±0.20	26.2±0.10

The laccase treatment on GA showed the highest oxygen consumption rate in the absence of pulp (Fig. 1), which was attributed to its three phenolic hydroxyls. The MS was also rapidly oxidized by laccase. This indicated that the syringyl-type MS and GA with more phenolic hydroxyl groups showed higher reactivity towards laccase. Therefore, they were easier to self-polymerize or graft on fibers via radical-coupling reactions. However, the SyA showed a lower activity as it carries an aldehyde group. This group has a strong electron-attracting effect and enhances the redox potential of SyA. This correlation between the reactivity and the component structure/function al groups is consistent with the results obtained by Mai and Schormann (2001), who studied the activity of laccase towards different phenolic compounds. For the HBA, it was the least reactive with laccase. Thus, it self-polymerizes less rapidly in the reaction conditions and has more opportunities to spread or deposit on the fiber surface, which supports the high COOH content with low brightness decrease.

Figure 2 shows that, compared with the laccase treatment of a compound, the oxygen consumption of the samples increased to a varying degree when laccase and the phenolic compound were added to the pulp (Fig. 2). The results indicated that the participation of all compounds enhanced the reactivity of pulp to laccase. The significant increase in oxygen consumption can be observed in the case of L/MS+P sample. The oxygen concentration decreased sharply and was exhausted within 4 minutes using MS or GA. Therefore, more self-polymerizations or graft reactions occurred on fibers, since a large quantity of phenoxy radicals were present in the reaction system. The self-polymers were deposited on the fiber surface and thus brought a higher opacity of handsheets (Table 1).

The grafting of GA with carboxyl groups on fibers contributed to a higher dry tensile strength. However, the L/SyA-treated samples with higher wet strength and dry strength had lower oxygen consumption. Moreover, compared with the oxygen consumption in Fig. 1, the oxygen consumption in Fig. 2 slightly increased. This shows that the SyA radical is very stable and has enough time to graft or mediate the lignin oxidation by laccase. Therefore, this reactivity level did not show a direct relation to the size of the paper strength.

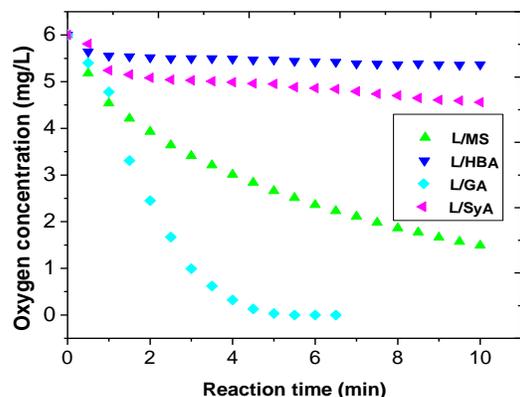


Fig. 1. Oxygen consumption during laccase treatment of different phenolic compounds

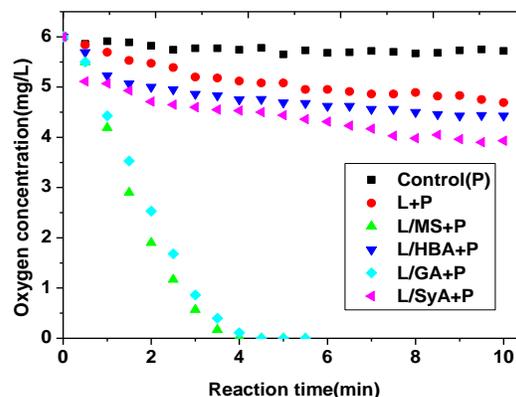


Fig. 2. Oxygen consumption during laccase treatment of pulp with different compounds

Fiber Quality Analysis

The fiber morphology has an important influence on the pulp strength. The fiber coarseness increased to a different degree after laccase treatment with all the compounds (Table 2). Especially, the fiber coarseness of fibers treated with GA and MS increased by 15.0% and 17.1%, respectively, which was caused by the polymerization/graft of GA and MS on the fiber surface. This theory also supports the higher opacity, lower brightness, and the observations from the oxygen consumption experiments.

Table 2. Fiber Morphology After Laccase(L) Treatment with Different Compounds

Treatments	Coarseness(mg/100m)	Curl index (Lw)	Kink index	Kink angle (°)
Control	14.0 ± 0.25	0.135 ± 0.05	1.96 ± 0.07	48.45 ± 0.16
Laccase	14.1 ± 0.30	0.114 ± 0.04	1.85 ± 0.05	46.21 ± 0.14
L/MS	16.4 ± 0.42	0.108 ± 0.08	1.81 ± 0.05	41.95 ± 0.18
L/HBA	14.8 ± 0.21	0.117 ± 0.07	1.83 ± 0.05	44.63 ± 0.20
L/GA	16.1 ± 0.41	0.104 ± 0.07	1.74 ± 0.06	40.77 ± 0.20
L/SyA	15.8 ± 0.36	0.109 ± 0.05	1.80 ± 0.05	42.85 ± 0.15

Fiber curl is the gradual and continuous curvature of a fiber. Fiber kink is an abrupt change in the curvature of a fiber and it is often caused by damage of the cell wall. The curliness and kink of fiber affect the tensile strength negatively. Table 2 shows that the curl index, kink index, and kink angle of fibers decreased to a different degree after laccase treatment with different compounds. It is suggested that the self-polymers or copolymers of compounds on the fiber surface can fill up the damaged cell wall and make the fibers stretch. Therefore, the decrease of curl index and kink index helps to increase the entanglement and contact joints between fibers, which result in an increase of the bonding areas.

XPS Analysis

X-ray photoelectron spectroscopy (XPS) has been shown to be a useful tool for the study of chemical structure of fiber surface. Table 1 shows that the L/MS-treated pulp had the largest wet strength and the L/HBA-treated sample had the largest dry strength.

Moreover, MS and HBA differ very much in chemical structure. Therefore, they were selected to be analyzed by XPS. The high-resolution XPS spectra of the C1s for the MS or HBA-treated fibers are shown in Fig. 3. The surface lignin and C1-C4 components of fibers are summarized in Table 3.

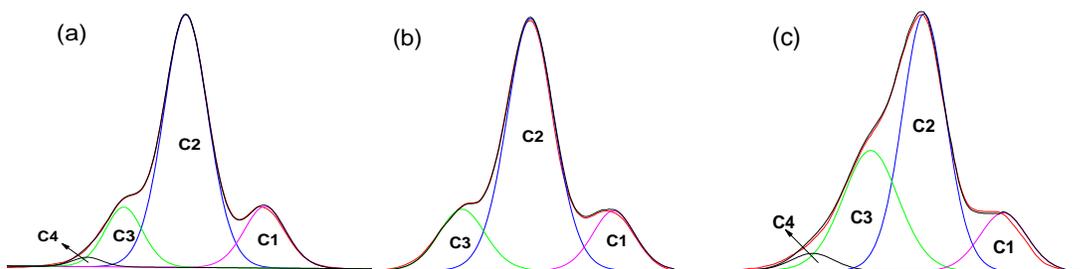


Fig. 3. High-resolution XPS spectrums of the C1s for the acetone-extracted fibers: (a) control, (b) laccase/MS-treated, (c) laccase/HBA-treated fibers

Table 3. Chemical Structure of Fiber Surface after Laccase (L) Treatment with MS or HBA

Treatment	C1s (%)	O1s (%)	O/C*	Surface lignin (%)	C1s total = 100%			
					C1 (%), {C-C, C-H}	C2 (%), {C-O}	C3 (%), {O-C-O or C=O}	C4 (%), {O-C=O}
Control	60.06 ± 0.40	39.94 ± 0.28	66.50 ± 0.30	33.0 ± 0.18	14.72 ± 0.17	69.79 ± 0.42	13.75 ± 0.14	1.73 ± 0.12
L/MS	61.15 ± 0.38	38.85 ± 0.30	63.53 ± 0.28	38.9 ± 0.20	15.53 ± 0.15	68.50 ± 0.45	15.97 ± 0.16	—
L/HBA	60.08 ± 0.38	39.92 ± 0.26	66.44 ± 0.28	33.1 ± 0.18	12.93 ± 0.18	52.42 ± 0.40	31.17 ± 0.18	3.48 ± 0.12

* The table shows atomic ratios of O/C in low-resolution survey scans and amount of C1 in high-resolution carbon spectra (carbon in different functional groups with oxygen).

It has been reported that laccase may catalyze polymerization of lignin fragments to surface lignin or de-polymerization of surface lignin, and this balance between these opposing mechanisms depends on the nature of mediators (Felby *et al.* 2004). Enzymatically catalyzed bonding is linked to the oxidative generation of stable radicals in lignin, and the radicals can cause cross-linking or loosening of the lignin structure (Felby *et al.* 1997). Table 3 shows that the surface lignin coverage of fibers significantly increased from 33.0% to 38.9% after laccase/MS treatment, and the kappa number slightly increased from 24.9 to 25.6. This indicates that the modification of fibers by laccase/MS occurred mainly on the surface of fibers. The MS is a lignin-related compound and the surface lignin of L/MS-treated pulp significantly increased. It was very likely that the MS was grafted on fiber surface via radical cross-linking reaction. According to the results of oxygen consumption and fiber quality analysis (FQA), the MS was easier to self-polymerize or graft on the fibers. Thus, the C1 component (C-C, C-H, related to the presence of lignin) increased from 14.72% to 15.53% after laccase/MS treatment. In addition, the C2 component (C-O, from cellulose hydroxyl) decreased from 69.79% to 68.50% and the C4 component (O-C=O, from carboxyl group) were too low to

be detected. These results could be caused by the coverage of the grafted/polymerized MS over hydrophilic hydroxyl and carboxyl group from carbohydrate. The increased C3 component (C=O) showed the oxidation of surface lignin.

For the L/HBA-treated fiber surface, the significant increase of C3 component and reduction of C2 could be observed. This might suggest the oxidation of the original pulp surface carbohydrates or the surface carbohydrate's covered. The C4 component, representing carboxyl on the fiber surface, increased from 1.73% to 3.48%, suggesting the graft of HBA on fibers. As shown in Fig. 1, the phenoxyl radical from HBA is very stable due to its low activity to laccase. On the other hand, it has a smaller steric hindrance. Therefore, it has more opportunity to react with the surface lignin and penetrate fiber pores. The phenolic moieties may participate in not only the oxidative degradation but also dehydrogenative polymerization reactions of residual lignin in the pulp by laccase (Balakshin *et al.* 2001). Tables 1 and 3 showed that the surface lignin coverage and kappa number of the L/HBA-treated fibers had little change. It is suggested that, in this work, the HBA might play the role of mediator in the laccase-catalyzed reaction.

AFM Analysis

The nanostructure and surface morphology of fibers can be observed with AFM, which provides a detailed view of fiber surface characteristics. The lignins appear as patches or granular phases on the surface of the extracted fiber, and the hemicelluloses are present in amorphous or fibrillar phases (Gustafsson *et al.* 2003). The light and amorphous matter in the higher phase region is the hydrophobic lignin; the dark and microfibrillar matter in the low phase region is the hydrophilic cellulose (Chernoff 1996).

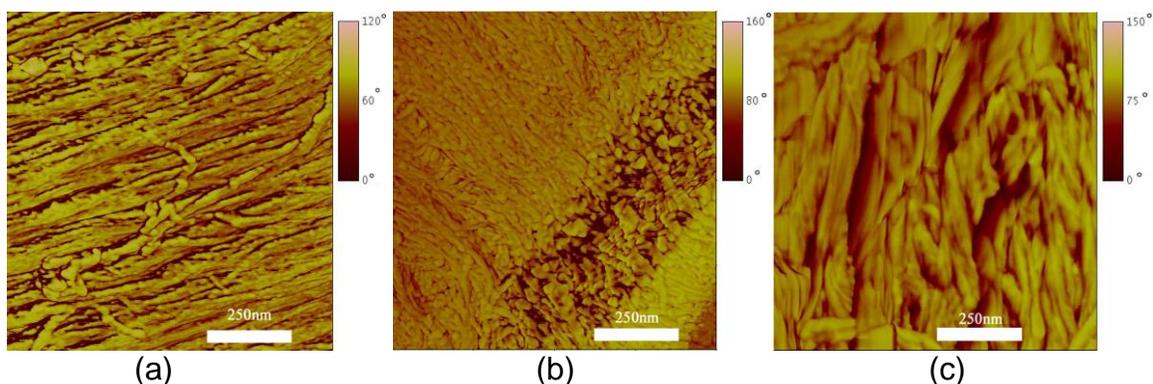


Fig. 4. Atomic force microscopy (AFM) phase images of fibers: (a) control, (b) laccase/MS-treated, (c) laccase/HBA-treated. Image sizes are: 1.0 μm \times 1.0 μm

It can be seen that linear and parallel microfibrils predominated on the fiber surface of control samples, which showed the feature of the S2 lamella in the cell wall (Fig. 4a). There were a lot of granular or amorphous structures with higher phase on the control fiber surface, which was considered to be lignin. The short fibrillar matter might be the hemicellulose linked with lignin, namely lignin and carbohydrate complex. The previous work (Liu *et al.* 2008) showed that the phenoxyl radical concentration of laccase/MS-treated fibers increased sharply at the initial stage and then decreased, and that the radical concentration in suspension of laccase treatment of MS exhibited a rapid

decay with time during the course of reaction. In this work, a majority of massive granules appeared in the wide area on the L/MS-treated fiber surface (Fig. 4b). This observation is in accord with an increase in fiber coarseness. These findings indicated that the polymerized MS by radical coupling deposited on the fiber surface, or that the MS was grafted on fiber surface by reacting with surface lignin. These polymers filled up the breakage of fiber surface (the dark region in Fig. 4b). The observation agrees with the decrease of kink index and curl indexes. This homogeneity of fiber surface is just another contributing explanation for the paper strength improvement.

As for the laccase/HBA-treated fibers (Fig. 4c), the granules representing lignin disappeared, and some gaps were observed on the fiber surface. The oxidation of the original pulp surface carbohydrates might occur according to the XPS results, which resulted in the loose fiber surface. The oxidated HBA with small steric hindrance is easier to penetrate into the fiber and mediate laccase-catalyzed reaction. Some big features on the fiber surface may be from the product of the graft polymerization reaction. In fact, the HBA is a lignin-derived phenol, so that the surface lignin and kappa number of fibers changed little (Table 1). It is suggested that the fiber modification with laccase/HBA mainly took place in the fibers, whereas the fiber modification with laccase/MS mainly occurred on the fiber surface. These results confirmed that the modification mechanism of fibers was related to the chemical structure of compounds, for example, the functional group and steric hindrance.

CONCLUSIONS

The syringyl-type phenols (e.g. MS and SyA) with hydrophobic group (OCH_3) are especially effective for improving the wet strength of pulp. The phenols with carboxyl groups help to enhance the dry strength of pulp. The coarseness of all the treated fibers increased, and the curl index and kink index of fibers decreased. The syringyl-type MS or GA with more phenolic hydroxyl group has a higher activity to laccase. The participation of phenolic compound also enhances the reactivity of fibers to laccase in varying degree. However, this reactivity level has no direct relation to the size of the paper strength. The enhancement of wet strength is probably related to the polymerization/copolymerization of phenols with hydrophobic group and information of covalent bonding via radical coupling. The attachment of carboxyl groups to fibers contribute to the improvement of dry strength. Moreover, the improvement mechanisms are also related to the increase in entanglement and interfacial homogeneity of fibers.

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