Fiber Modification of Unbleached Kraft Pulp with Laccase in the Presence of Ferulic Acid

Hui Li,[a] Shiyu Fu,[b,*] and Lincai Peng [a]

Unbleached kraft pulp fibers were modified with laccase and ferulic acid (FRC) to improve their physical strength properties in paper products. The optimal conditions of laccase-FRC modification were examined in terms of the physical properties of pulps. The effects of laccase-FRC modification on the carboxyl group content and surface lignin content of pulps were investigated. The surface morphologies of laccase-FRC-modified pulp fibers were observed by atomic force microscopy (AFM). The carboxyl group and surface lignin contents for laccase-FRC-modified pulps increased compared to the control pulp. AFM phase images showed that the laccase-FRC-modified fiber surfaces were covered with large granular substances from the products of FRC grafting and lignin polymerization/condensation reactions. The observed strength improvements of laccase-FRC-modified pulp could be attributed to the grafting of FRC onto the fibers, the higher carboxyl group content of the modified fibers, and the formation of covalent bonding between the fibers *via* radical coupling.

Keywords: Laccase; Ferulic acid; Kraft pulp; Physical properties; Fiber modification

Contact information: a: Faculty of Chemical Engineering, Kunming University of Science and Technology, Kunming 650500, China; b: State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China;
** Corresponding authors: shyfu@scut.edu.cn*

INTRODUCTION

Enzymatic fiber modification has been established as an important area of research for improving the processing and properties of lignocellulosic materials (Park *et al.* 2006). Chemical modification involves harsh reaction conditions, loss of desirable components, and the potential use of hazardous chemicals, whereas enzymatic modification conditions are often milder, less damaging for the fibers, and more environmentally compatible (Zhang *et al.* 2013; Aracri *et al.* 2011). Enzymatic modification of fibers can be achieved by glucohydrolysis and oxidative enzymes (Kenealy *et al.* 2006). One notable oxidative enzyme is laccase, which is a multi-copper-containing oxidoreductase. This particular oxidoreductase catalyzes the oxidation of phenolic compounds, aminophenols, polyphenols, polyamines, and lignin-related molecules while concomitantly reducing oxygen to water (Couto and Herrera 2006).

In the last few decades, laccase has been widely used in the pulp and paper industry for pulp biobleaching (Fillat and Roncero 2009). Although there have been many thorough investigations of the potential applications of laccase in the biobleaching process (Aracri *et al.* 2012; Fillat *et al.* 2010), the current focus on laccase has shifted toward pulp fiber modification (Liu *et al.* 2013). Laccase and laccase-mediator systems can modify the fiber with a view to improve its chemical or physical properties by enzymatic activation of high lignin-containing fibers (Mocciutti *et al.* 2008; Chen *et al.*

2012). Lund and Felby (2001) found that wet tensile strength was significantly improved when the unbleached high-yield kraft pulp was modified with a laccase-mediator system. It has been reported that laccase oxidation of pulp fibers increased the amount of carboxyl groups contained on the original fibers (Mohandass *et al.* 2008). This modification was shown to improve fiber swelling and flexibility, which may be beneficial in the bonding of pulp fibers in paper to increase paper strength (Witayakran and Ragauskas 2009). Chen *et al.* (2012) showed that the carboxyl group content and physical strength properties of old corrugated container pulp increased when modified with laccase or a laccase-HBT (1-hydroxybenzotriazole) system.

In addition, laccase has been shown to have the potential to graft several low-molecular weight compounds onto high lignin content pulp fibers. These grafts improved physical strength or imparted new properties to the pulp fibers (Chandra *et al.* 2004; Liu *et al.* 2009; Chen *et al.* 2010; Aracri *et al.* 2011; Fillat *et al.* 2012). Chandra *et al.* (2004) found that the burst and tensile strengths of high-kappa kraft pulp increased when gallic acid was grafted onto pulp fibers with laccase. Liu *et al.* (2009) showed that the wet tensile strength of unbleached kraft pulp modified with laccase-syringate was twice as high as that modified with laccase. Chen *et al.* (2010) reported laccase-mediated grafting of histidine onto old newspaper pulp fibers; the authors found an increase in the carboxyl group content and tensile strength of the treated pulp. Fillat *et al.* (2012) successfully prepared antimicrobial paper through the grafting of low molecular weight phenols onto unbleached flax fibers by laccase treatment.

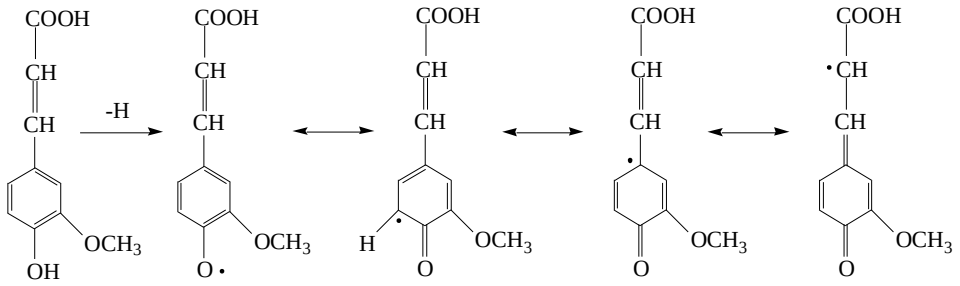

Fig. 1. Formation of free radicals from ferulic acid catalyzed by laccase

Ferulic acid (FRC) is a low-molecular weight phenolic compound. Compared with other low-molecular weight phenols such as vanillic acid, syringate, and gallic acid, FRC should be easy to graft to the fibers catalyzed by laccase because of conjugated –C_α=C_β–structure on slide chain with benzyl rings which can make FRC form free radicals easily (Fig. 1). In this study, unbleached kraft pulp was modified with laccase and FRC in an attempt to enhance its physical strength properties. The modification of pulp properties was evaluated by measuring changes in the physical and chemical properties of pulp fibers. The optimal laccase-FRC modification conditions with respect to the physical strength properties of pulp were investigated. The effects of laccase-FRC modification on the carboxyl group content and the surface lignin content of pulp were also examined. The effects of laccase-FRC treatment on the surface morphologies of pulp fibers were studied by AFM (atomic force microscopy). The aim of this study was to obtain a detailed understanding of fiber modification by laccase in the presence of FRC.

EXPERIMENTAL

Materials

Unbleached eucalyptus kraft pulp with a kappa number of 35.2 was prepared with a kraft cooking process. Prior to modification, the pulp was refined using a PFI mill until its beating degree was 45ºSR. Laccase produced by *Flammulina velutipes* (Fv) and analytical grade FRC were purchased from Shanghai Aladdin Reagent Co., Ltd (Shanghai, China). The laccase activity was determined by monitoring the oxidation rate of 2,2'-azinobis-(3-ethylbenzyl thiozoline-6-sulfonate) (ABTS) according to Mansfield (2002). 1 IU of laccase activity is defined as the amount of laccase that oxidizes 1 μmol of ABTS substrate per minute. The laccase activity used in this study is 20 IU/mL.

Enzymatic Modification of Pulp Fibers

Laccase (0, 5, 15, 25, and 35 IU/g) and FRC (0%, 0.5%, 1.0%, 1.5%, and 2.0% on dry pulp) were mixed with eucalyptus kraft pulp (5% consistency) buffered at various pH levels (3.0, 4.0, 5.0, and 6.0) with 50 mM sodium acetate buffer. The pulp slurry was magnetically stirred in a water bath at 50 ºC under an oxygen atmosphere (continuous bubbling) for various reaction times (1, 1.5, 2, and 2.5 h). After modification, the pulp samples were filtered and washed with deionized water until the filtrate was colorless. Pulp samples were treated under the same conditions but without both laccase and FRC were used as controls.

Handsheet Formation and Paper Testing

Handsheets with a grammage of 100 g/m^2 were made using a semiautomatic sheet former equipped with circulation water. Prior to paper testing, the handsheets were kept at constant temperature and humidity (23 ºC, 50% relative humidity) for at least 24 h. The dry tensile strength, wet tensile strength, tear strength, and burst strength were measured according to TAPPI methods T494, T456, T414, and T403, respectively.

Analysis of Pulp Properties

Determination of the carboxyl group content of pulp was performed in accordance with TAPPI standard T237. The detailed procedure was described by Chen *et al.* (2012). The reported results are the average of three measurements. Acid-insoluble (Klason lignin) lignin was determined according to T 249.

X-ray Photoelectron Spectrometer (XPS) Analysis

The surface chemical composition of samples was analyzed by XPS (Axis Ultra DLD, Kratos Analytical, UK) equipped with a monochromatic A1 *Kα* X-ray source, operated at 150 W, and the pass energy was 40 and 160 eV for high- and low-resolution spectra, respectively. A Gaussian curve fitting program was used to deconvolute the C1 carbon (C-C, C-H and C=C functional groups) signal at 284.6 eV. The chemical shifts relative to C1 used in the deconvolution were 1.7±0.2 eV for C2(C-O), 3.1±0.2 eV for C3(C=O or O-C-O), and 4.3±0.2 eV for C4 (O-C=O). All samples before XPS analysis were subjected to acetone extraction to remove pulp extractives. The surface lignin content of the fibers was calculated from the O/C atomic ratios using the equation described by Ström and Carlsson (1992),

$$\phi_{lignin} = \frac{(O/C)_{pulp-sample_{after-extraction}} - (O/C)_{pure-pulp}}{(O/C)_{lignin} - (O/C)_{pure-pulp}} \quad (1)$$

where ϕ_{lignin} represents the surface lignin content of the pulp fiber. Values of 0.33 and 0.83 were used for $(O/C)_{lignin}$ and $(O/C)_{pure\text{-}pulp}$, respectively, according to Ström and Carlsson (1992).

Atomic Force Microscopy (AFM) Analysis

The surface morphologies of control and modified fibers were observed using a Nanoscope IIIa AFM (Veeco Co., USA). The samples for AFM were those used for XPS analysis. The instrument was operated in tapping mode with silicon cantilever tips. No image processing except flattening was made. The scan size was 2 μm× 2 μm. Software version 5.12r3 (Veeco Co., USA) was used for online recording and offline analysis.

RESULTS AND DISCUSSION

Effect of Laccase-FRC Modification Conditions on the Strength Properties of Pulp

Table 1 shows the effect of laccase dose on the physical properties of pulp. Compared to the control sample, all the physical strength indices of the pulp increased with increasing laccase dose. When the laccase dose was 15 IU/g, the dry and wet tensile indices reached their maximum value, increasing by 4.2% and 56.9% compared to the control, respectively. Meanwhile, both the tear and burst indices reached their optimal values, increasing by 6.5% and 3.7%, respectively, compared to the control. When the laccase dose exceeded 15 IU/g, the dry tensile index showed little change and the wet tensile index decreased. Therefore, the optimal laccase dose for laccase-FRC modification was judged to be 15 IU/g.

Table 1. Effect of Laccase Dose on the Physical Properties of Pulp

Laccase dose (IU/g)	Dry Tensile Index (Nm/g)	Wet Tensile Index (Nm/g)	Tear Index ($mN•m^2/g$)	Burst Index ($kPa•m^2/g$)
control	81.43	2.58	8.46	4.89
0	81.58	2.61	8.23	4.90
5	82.66	3.97	8.93	5.02
15	84.87	4.05	9.01	5.07
25	84.72	3.58	9.15	5.13
35	84.58	3.14	9.11	5.08

FRC dose 1%, treatment time 2.0 h, pH 4.0

The increasing degree of wet tensile strength was higher than that of dry tensile strength, which is consistent with the findings of Aracri *et al.* (2011), who studied the effects of laccase-catalysed grafting of ferulic acid on physical properties of sisal pulp fibers (Table 1). It was reported that dry tensile strength in paper originates mainly from a large number of hydrogen bonds between adjacent fibers, whereas wet tensile strength is related to bonds being resistant to water sorption like covalent and ionic bonds or creation of a polymer matrix around fibers (Lund and Felby 2001). The reason why the increment

of dry tensile strength was lower than wet tensile strength is probably due to lower increased inter-fiber bonding levels and there was not enough to cause the large improvement of dry tensile strength, whereas the minor increase in bonding has a significant positive impact on wet tensile strength at low levels of bonding (Page 1969). In addition, it is worth noting that the tear and burst indices of modified pulps were slightly improved compared to the control. Chandra *et al* (2004) reported that the burst, dry tensile, and wet tensile strength of laccase-gallic acid modified high kappa number (91) kraft pulp increased by 34%, 20%, and 72%, respectively, compared to the control. The authors concluded that lignin was the major target for the fiber modification. The kappa number of kraft pulp used in this study is 35.2, and we assumed that the relative low lignin content is the main reason for the small increase in tear and burst strength properties.

Table 2 shows the effect of FRC dose on the physical properties of the pulp. The dry tensile, wet tensile, tear, and burst indices of the pulp all improved with the increase in FRC dose. The dry tensile, wet tensile, and burst indices reached their maximum values when the FRC dose was 1%. When the FRC dose exceeded 1%, there was little change in both the dry tensile and wet tensile indices. Therefore, 1% was chosen as the optimal FRC dose for laccase-FRC modification. Moreover, compared to the control sample, the dry tensile, wet tensile, tear, and burst indices of the pulp modified with laccase alone (15 IU/g laccase dose) increased by 2.0%, 5.8%, 1.8%, and 1.4%, respectively, while these indices of pulp modified with laccase-FRC (1% FRC and 15 IU/g laccase dose) increased by 4.1%, 56.9%, 6.5%, and 3.7%, respectively. These results indicated that the physical properties of pulp were significantly improved when modified with laccase and FRC.

Table 2. Effect of FRC Dose on the Physical Properties of Pulp

FRC dose (%)	Dry Tensile Index (Nm/g)	Wet Tensile Index (Nm/g)	Tear Index ($mN•m^2/g$)	Burst Index ($kPa• m^2/g$)
control	81.43	2.58	8.46	4.89
0	82.09	2.73	8.61	4.96
0.5	83.13	3.54	8.87	5.05
1.0	84.87	4.05	9.01	5.07
1.5	84.51	4.01	9.13	5.01
2.0	83.76	4.02	9.07	4.98

Laccase dose 15 IU/g, treatment time 2.0 h, pH 4.0

Table 3 illustrates the effect of treatment time on pulp physical strength. When the treatment time increased from 1.0 to 2.0 h, the dry tensile, wet tensile, tear, and burst indices increased by 1.4%, 13.1%, 2.1%, and 2.0%, respectively. However, the changes in the dry tensile, wet tensile, tear, and burst indices were small when the treatment time exceeded 2.0 h. Therefore, the optimal treatment time for laccase-FRC modification was 2.0 h.

The effect of reaction pH is summarized in Table 4. It can be seen from this table that there is almost no obvious change in the dry tensile index, whereas the wet tensile index increased with increasing reaction pH. The reaction pH was 4.0, the wet tensile index obtained its maximum value and the tear and burst indices leveled-off at their maximum values. Therefore, the optimal reaction pH was 4.0.

Table 3. Effect of Treatment Time on the Physical Properties of Pulp

Time (h)	Dry Tensile Index (Nm/g)	Wet Tensile Index (Nm/g)	Tear Index ($mN•m^2/g$)	Burst Index ($kPa•m^2/g$)
control	81.43	2.58	8.46	4.89
1.0	83.71	3.58	8.83	4.97
1.5	84.69	4.11	8.79	5.06
2.0	84.87	4.05	9.01	5.07
2.5	83.51	4.08	8.94	5.03

Laccase dose 15 IU/g, FRC dose 1%, pH 4.0

Table 4. Effect of Reaction pH on the Physical Properties of Pulp

pH	Dry Tensile Index (Nm/g)	Wet Tensile Index (Nm/g)	Tear Index ($mN•m^2/g$)	Burst Index ($kPa•m^2/g$)
control	81.43	2.58	8.46	4.89
3.0	84.65	3.34	8.53	5.02
4.0	84.87	4.05	9.01	5.07
5.0	84.11	3.88	9.13	5.04
6.0	84.69	3.93	8.92	5.11

Laccase dose 15 IU/g, FRC dose 1%, treatment time 2.0 h

Effect of Laccase-FRC Modification on the Carboxyl Group Content and lignin content of Pulps

The above experimental results showed that the optimal physical properties of pulp were obtained when the modification conditions were laccase dose 15 IU/g, FRC dose 1%, reaction time 2.0 h, and pH 4.0. Therefore, these reaction conditions were chosen to investigate the impact of laccase-FRC modification on fiber chemistry.

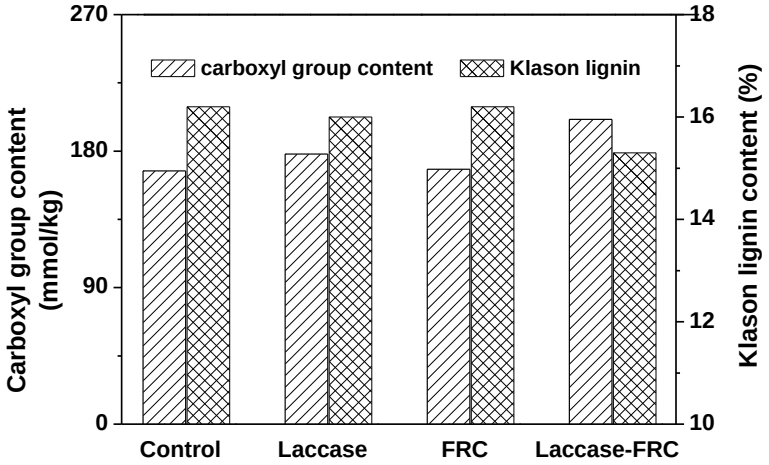

Fig. 2. Carboxyl group contents and klason lignin contents of control and modified pulps

Carboxyl groups are beneficial in the bonding of pulp fibers in paper, which contributes to paper strength. The carboxyl group contents for control and modified pulps are shown in Fig. 2. It can be seen that the laccase-modified pulp had higher carboxyl group content than the control pulp, which is due to the oxidation of lignin by laccase (Shleev *et al.* 2006; Betcheva *et al.* 2007). This was in accordance with the study results of Chen *et al.* (2012), who reported an increase in carboxyl group content for laccase-

modified old corrugated container pulp. In addition, FRC-modified pulp yielded similar carboxyl group content when compared to the control pulp, which suggested that FRC itself did not react with the lignin in the pulp fibers under the reaction conditions employed. However, when the pulp was modified with both laccase and FRC, the modified pulp provided the highest carboxyl group content. These results indicated that laccase facilitated the grafting of FRC onto lignin-rich fibers, similar to the description by Witayakran and Ragauskas (2009). In addition, it cannot be ruled out that lignin was oxidized in the presence of laccase-FRC, resulting in the increased carboxyl group content. The carboxyl group content of laccase-FRC-modified pulp increased by 20.4% compared to the control sample. The increase in carboxyl group content facilitated the bonding of the pulp fibers, resulting in increased paper strength, which is in accordance with the above experimental results (Tables 1- 4). Furthermore, the Klason lignin contents of control and modified pulps are also presented in Fig. 2. The results indicate that laccase-FRC modified pulp had a slight reduction in Klason lignin content when compared to the control pulp, which is due to the delignification of laccase-FRC system. The decrease in lignin content resulted in increasing flexibility of pulp, thus resulting in strength properties improvement of pulp.

Effect of Laccase-FRC Modification on the Surface Lignin Content of Pulp

The surface chemistry of lignocellulosic fiber materials significantly affects the fiber characteristics and papermaking process. The surface chemical composition of the pulp may influence its bonding properties, including adhesion and strength, and its optical properties (Šernek *et al*. 2004). To further investigate the change in surface chemistry of the fibers after laccase and laccase-FRC modification, the surface lignin contents of control and modified fibers were examined using XPS. XPS analysis results for the control and modified fibers are illustrated in Fig. 3 and listed in Table 5.

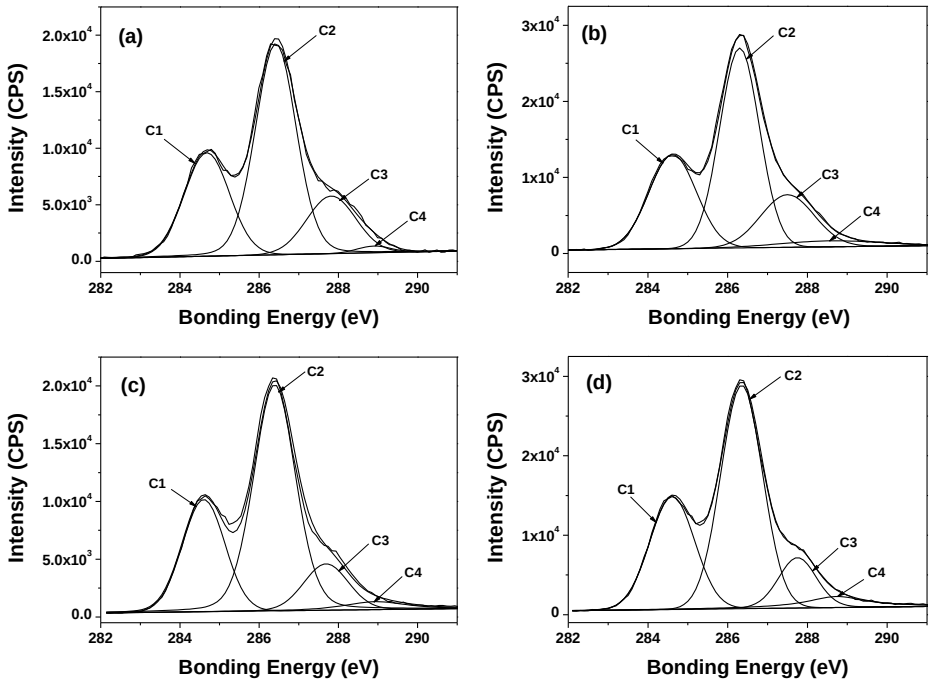

Fig. 3. High-resolution spectra of the C1s peak for control and modified fibers: (a) control fiber, (b) laccase-modified fiber, (c) FRC-modified fiber, and (d) laccase-FRC-modified fiber

Table 5. XPS Analysis Results for Control and Modified Fibers

Pulp sample	O/C	ϕ_{lignin}	C 1s total = 100%			
			C1	C2	C3	C4
Control	0.568	52.4	27.7	54.3	16.6	1.4
Laccase	0.553	55.4	29.1	47.9	21.3	1.7
FRC	0.567	52.5	27.8	54.5	16.3	1.4
Laccase-FRC	0.532	59.6	31.2	44.6	21.8	2.4

As shown in Table 5, the surface lignin content of the laccase-modified fibers was higher than that of the control fiber, which indicated the condensation of surface lignin due to laccase (see Fig. 4a–4b). The condensed lignin on the fiber surface modified with laccase may act as a wet strength agent during paper forming, which could be one explanation for the paper wet strength improvement (Lund and Felby 2001). For the FRC-modified fiber, the surface lignin content of the fibers was nearly identical to that of the control fibers, which meant that there was no change in fiber surface chemistry. For the laccase-FRC-modified fiber, the surface lignin content was higher than that of the control fiber and the laccase-modified fiber. The structure of FRC used in this work is similar to phenolic end units in lignin. Laccase not only can catalyze lignin on the fiber surface to form lignin phenoxy radicals, but it also can catalyze FRC to form radicals. Therefore, when pulp is modified with laccase and FRC, FRC radicals can couple with lignin radicals on the fiber surface and form a FRC-lignin complex, thereby resulting in an increase in surface lignin content (see Fig. 4c–4d).

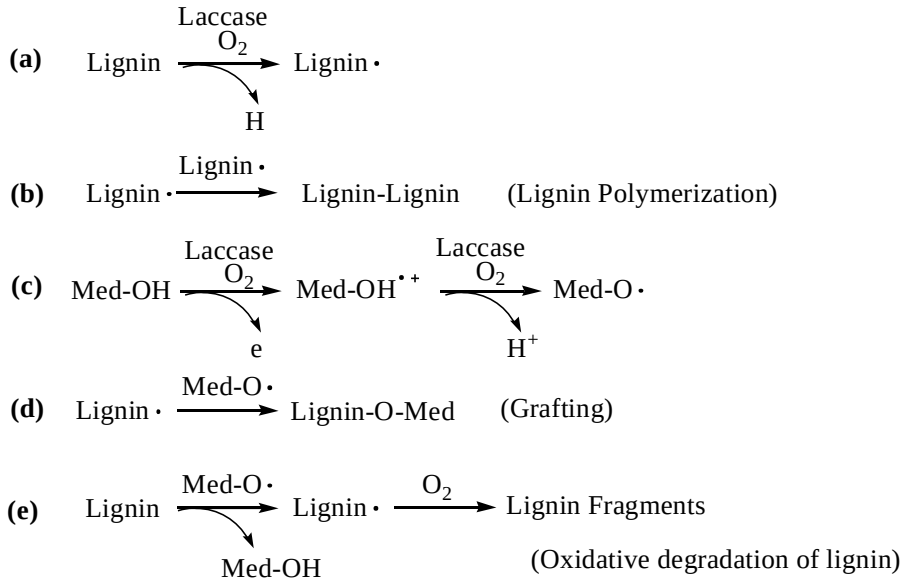

Fig. 4. Chemical processes in laccase alone and laccase-FRC modification. Med-OH represents the mediator FRC.

Lund and Felby (2001) found that laccase could catalytically polymerize lignin fragments onto surface lignin or depolymerize lignin *via* oxidation in the presence of a mediator. The balance between these two opposing mechanisms depends on the nature of the redox mediator used. Barneto *et al.* (2012) reported that FRC acted as a natural mediator for laccase biobleaching to effectively delignify sisal pulp. In this study, we

propose that laccase catalyzed the oxidation of FRC into FRC radicals, which have minimum steric hindrances and can more easily penetrate into the fiber; these FRC radicals mediate the laccase-catalyzed reaction (see Fig. 4e), and this chemical process was verified by the above Klason lignin content measurement. Thus, another possible contribution to the increase in the surface lignin content of laccase-FRC-modified fiber is that the degradation of lignin in fibers during laccase-FRC modification process makes the fiber cell wall structure loose, and then degraded lignin fragments are easily released from the fiber cell wall, which polymerized and precipitated on the fiber surface (Lund and Felby 2001). This is in a manner similar to added lignin as described by Elegir *et al.* (2007), who used laccase and ultra-filter lignin to improve the mechanical properties of kraft liner pulp. Therefore, the FRC used in this study may have acted not only as a graft monomer onto the lignin, but also as a laccase mediator.

According to Felby *et al.* (1997), the observed strength improvement of laccase-modified pulp could also be ascribed to the coupling of phenoxyl radicals of lignin associated with adjacent fiber surfaces. The FRC-mediated oxidation not only enhanced the generation of phenoxy radicals on the surface lignin *versus* laccase alone, but also grafted FRC onto the fiber, which resulted in the increased carboxyl group content. These are possible explanations for the strength improvements of laccase-FRC-modified pulp. The laccase-FRC modification of kraft brownstocks could improve the pulp's physical strength, making it useful for numerous packaging products subjected to different types of mechanical stresses.

As shown in Table 5, the C1 component of the pulp modified with laccase alone increased from 27.7% (control sample) to 29.1% due to the polymerization of surface lignin. The C2 component of pulp modified with laccase alone was lower than that of the control sample, which could be attributed to the coverage of condensed lignin over hydroxyl from carbohydrate (Liu e*t al.* 2009). Meanwhile, increases in the C3 and C4 components of pulps modified with laccase alone were also found. These changes were caused by the oxidation of lignin by laccase, which resulted in the increase in carbonyl and carboxyl groups. For the FRC-modified pulp, the concentrations of the C1-C4 components on the fiber surface remained constant, which further demonstrated that FRC itself cannot be grafted onto the fiber surface and did not modify the fiber surface. For the laccase-FRC-modified pulp, the C1 component increased from 27.7% (control sample) to 31.2%, the C2 component decreased from 54.3% to 44.6%, and the C4 component increased from 1.4% to 2.4%. These results could be caused by the coverage of polymerized lignin and grafted FRC over hydroxyl from carbohydrate. The significant increased C3 component showed that FRC accelerated the oxidation of lignin by laccase.

Surface Morphology Analysis of Fiber Modified with Laccase-FRC

To further understand the effect of laccase-FRC modification on the surface characteristics of the fibers, the fiber surfaces of the control and modified fibers were observed using AFM. The surface morphological images of control and modified fibers are shown in Fig. 5. The control fiber surface was fully covered with granular substances (Fig. 5a) and had a root-mean-square (RMS) roughness of 21.34 nm. The diameters of these granular substances were approximately 55 nm to 70 nm. These granular substances were considered to be lignin because Gustafsson *et al.* (2003) revealed that lignin appeared as patches or granular phases on the surface of the extracted fiber. For the FRC-modified fiber (Fig. 5b), the surface characteristics of fibers were similar to that of

control fibers. When the fibers were modified with laccase alone, large granular substances with diameters of 96 nm to 220 nm appeared on the fiber surfaces (Fig. 5c). Combining the AFM images and XPS results, we assumed that these larger granular substances were products of lignin polymerization/condensation. Larger granular substances were observed when the fiber was modified with laccase-FRC (Fig. 5d); these larger granular substances may consist of condensed lignin and/or FRC-lignin complex. The increased amount of large granular substances was in agreement with the increase in surface lignin content determined by XPS analysis (Table 5). The appearance of these large granular substances resulted in an increase in the RMS roughness of fiber surfaces. The surfaces of fibers modified with laccase alone and laccase-FRC had RMS roughness values of 27.17 nm and 28.43 nm, respectively. The increased RMS surface roughness of pulp fibers modified with laccase and laccase-FRC could enhance the bonding between fibers, thus resulting in the better paper strength properties.

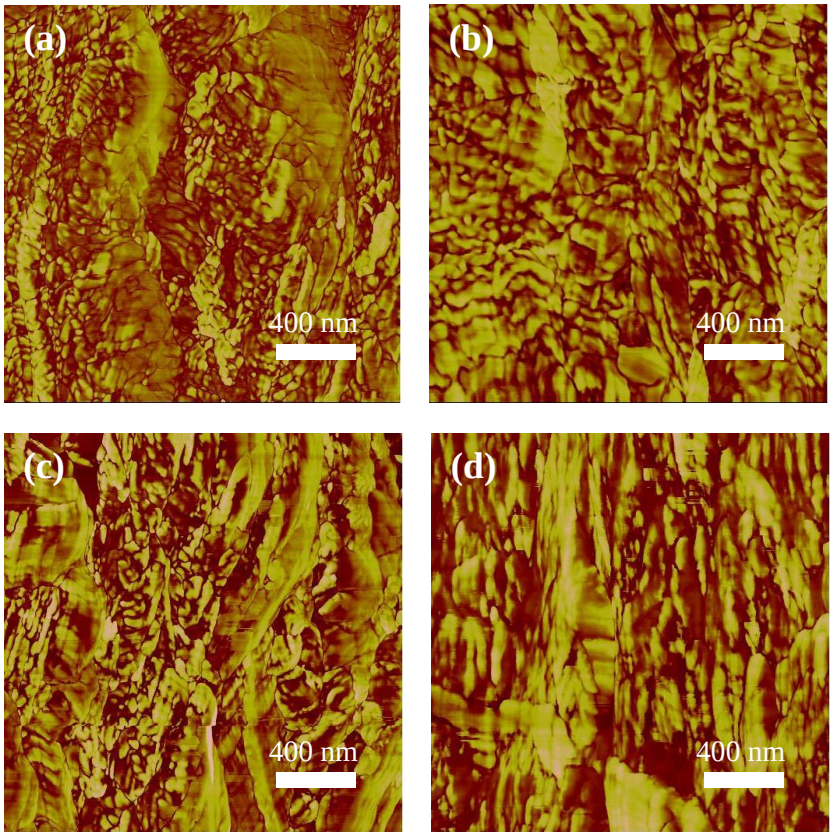

Fig. 5. AFM phase images of the control and modified fibers: (a) control fiber, (b) FRC-modified fiber, (c) laccase-modified fiber, and (d) laccase-FRC-modified fiber

CONCLUSIONS

1. The physical strength properties of unbleached kraft pulps improved after laccase-FRC modification.

2. The laccase-FRC-modified pulp yielded a 20.4% increase in carboxyl group content when compared to the control pulp; the increase in carboxyl groups facilitated bonding between the modified fibers and resulted in increased paper strength.
3. The surface lignin content of laccase-FRC-modified fibers was higher than that of laccase-modified fibers, indicating the simultaneous polymerization/condensation and degradation of the lignin during the laccase-FRC modification. AFM phase images showed that the surfaces of laccase-FRC-modified fibers were covered with large granular substance from the products of the grafting and lignin polymerization/condensation reactions.
4. Strength improvements of laccase-FRC-modified pulp could be attributed to the grafting of FRC onto the fibers, increased carboxyl group content of the modified fibers, and the formation of covalent bonding between the fibers via radical coupling.

ACKNOWLEDGEMENTS

This work was supported by Doctoral Program Foundation of Institutions of Higher Education of China (Grant No. 20090172110022), State Key Laboratory Open Foundation of Pulp and Paper Engineering of China (Grant No. 201225), and Talent Training Program of Yunnan Province (Grant No. KKSY201305002).

REFERENCES CITED

Aracri, E., Barneto, A. G., and Vidal, T. (2012). "Comparative study of the effects induced by different laccase-based systems on sisal cellulose fibers," *Ind. Eng. Chem. Res.* 51(10), 3895-3902.

Aracri, E., Roncero, M. B., and Vidal, T. (2011). "Studying the effects of laccase-catalysed grafting of ferulic acid on sisal pulp fibers," *Bioresour. Technol.* 102(16), 7555-7560.

Barneto, A. G., Aracri, E., Andreu, G., and Vidal, T. (2012). "Investigating the structure-effect relationships of various natural phenols used as laccase mediators in the biobleaching of kenaf and sisal pulps," *Bioresour. Technol.* 112, 327-335.

Betcheva, R. I., Hadzhiyska, H. A., Georgieva, N. V, and Yotova, L. K. (2007). "Biobleaching of flax by degradation of lignin with laccase," *BioResources* 2(1), 58-65.

Chandra, R. P., Lehtonen, L. K., and Ragauskas, A. J. (2004). "Modification of high lignin content kraft pulps with laccase to improve paper strength properties. 1. Laccase treatment in the presence of gallic acid," *Biotechnol. Progr.* 20(1), 255-261.

Chen, Y. M., Wan, J. Q., Ma, Y. W., and Lv, H. L. (2010). "Modification of properties of old newspaper with biological method," *Bioresour. Technol.* 101(18), 7041-7045.

Chen, Y. M., Wan, J. Q., Ma, Y. W., Tang, B., Han, W. J., and Ragauskas, A. J. (2012). "Modification of old corrugated container pulp with laccase and laccase-mediator system," *Bioresour. Technol.* 110, 297-301.

Couto, S. R., and Herrera, J. L. T. (2006). "Industrial and biotechnological applications of laccases: A review," *Biotechnol. Adv.* 24(5), 500-513.

Elegir, G., Bussini, D., Antonsson, S., Lindstrom, M. E., and Zoia, L. (2007). "Laccase-initiated cross-linking of lignocellulose fibres using an ultra-filtered lignin isolated from kraft black liquor," *Appl. Microbiol. Biotechnol.* 77(4), 809-817.

Felby, C., Nielsen, B. R., Olesen, P. O., and Skibsted, L. H. (1997). "Identification and quantification of radical reaction intermediates by electron spin resonance spectrometry of laccase-catalysed oxidation of wood fibers from beech (*Fagus sylvatica*)," *Appl. Microbiol. Biotechnol.* 48(4), 459-464.

Fillat, A., Colom, J. F., and Vidal, T. (2010). "A new approach to the biobleaching of flax pulp with laccase using natural mediators," *Bioresour. Technol.* 101(11), 4104-4110.

Fillat, A., Gallardo, O., Vidal, T., Pastor, F. I. J., Díaz, P., and Roncero, M. B. (2012). "Enzymatic grafting of natural phenols to flax fibres: Development of antimicrobial properties," *Carbohydr. Polym.* 87(1), 146-152.

Fillat, U., and Roncero, M. B. (2009). "Biobleaching of high quality pulps with laccase mediator system: Influence of treatment time and oxygen supply," *Biochem. Eng. J.* 44(2-3), 193-198.

Gustafsson, J., Ciovica, L., and Peltonen, J. (2003). "The ultrastructure of spruce kraft pulps studied by atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS)," *Polymer* 44(3), 661-670.

Kenealy, W., Buschle-Diller, G., and Ren, X. (2006). *Enzymatic Modification of Fibers for Textile and Forest Products Industries*, J. V. Edwards, G. Buschle-Diller, and S. C. Goheen (eds.), Springer, Netherlands.

Liu, N., Qing, M. H., and Li, Z. Q. (2013). "Laccase-catalyzed fiber functionalization with different phenolic compounds for enhancing pulp strength," *BioResources* 8(1), 887-899.

Liu, N., Shi, S. L., Gao, Y., and Qin, M. H. (2009). "Fiber modification of kraft pulp with laccase in presence of methyl syringate," *Enzyme Microb. Technol.* 44(2), 89-95.

Lund, M., and Felby, C. (2001). "Wet strength improvement of unbleached kraft pulp through laccase catalyzed oxidation," *Enzyme Microb. Technol.* 28(9-10), 760-765.

Mansfield, S. D. (2002). "Laccase impregnation during mechanical pulp processing improved refining efficiency and sheet strength," *Appita J.* 55(1), 49-53.

Mocciutti, P., Zanuttini, M., Kruus, K., and Suurnakki, A. (2008). "Improvement of the fiber-bonding capacity of unbleached recycled pulp by the laccase/mediator system," *TAPPI J.* 7(10), 17-22.

Mohandass, C., Knutson, K., and Ragauskas, A. J. (2008). "Laccase treatment of recycled blue dyed paper: Physical properties and fiber charge," *J. Ind. Microbiol. Biotechnol.* 35(10), 1103-1108.

Page, D. H. (1969). "A theory for the tensile strength of paper," *TAPPI J*. 52(4), 674-681.

Park, S., Venditti, R. A., Abrecht, D. G., Jameel, H., Pawlak, J. J., and Lee, J. M., (2006). "Surface and pore structure modification of cellulose fibers through cellulase treatment," *J. Appl. Polym. Sci*. 103(6), 3833-3839.

Šernek, M., Kamke, F. A., and Glasser, W. G. (2004). "Comparative analysis of inactivated wood surface," *Holzforschung* 58(1), 22-31.

Shleev, S., Persson, P., Shumakovich, G., Mazhugo, Y., Yaropolov, A., Ruzgas, T., and Gorton, L. (2006). "Interaction of fungal laccases and laccase-mediator systems with lignin," *Enzyme Microb. Technol.* 39(4), 841-847.

Ström, G., and Carlsson, G. (1992). "Wettability of kraft pulps-Effect of surface composition and oxygen plasma treatment," *J. Adhes. Sci. Technol.* 6(6), 745-761.

Witayakran, S., and Ragauskas, A. J. (2009). "Modification of high-lignin softwood kraft pulp with laccase and amino acids," *Enzyme Microb. Technol.* 44(3), 176-181.

Zhang, X. Y., Tan, J., Wei, X. H., and Wang, L. J. (2013). "Removal of Remazol turquoise blue G-133 from aqueous solution using modified waste newspaper fiber," *Carbohydr. Polym.* 92(2), 1497-1502.

Article submitted: June 21, 2013; Peer review completed: September 11, 2013; Revised version received and accepted: September 18, 2013; Published: September 26, 2013.