The Effect of Laccase Pretreatment Conditions on the Mechanical Properties of Binderless Fiberboards with Wheat Straw

Zexun Yang, Wei Song, Yang Cao, Cuicui Wang, Xiaoxia Hu, Yang Yang, and Shuangbao Zhang *

Self-bonding technology is potentially an effective solution to overcome formaldehyde emissions, which pose health and environmental concerns. Laccases can activate the fiber surface during the binderless fiberboard manufacturing process. This paper adopted wheat straw fibers (WSF) as the main raw material. The purpose of this study was to examine the effects of laccase pretreatment conditions on the mechanical properties of binderless fiberboards produced from WSF. For the improvement of mechanical properties, bamboo fibers (BF) were added as a reinforcing material. In addition, differences in the effects of two processes for adding laccase on the mechanical properties were monitored. As a result, binderless fiberboards were successfully manufactured from laccase-treated WSF. The results showed that the optimized pretreatment conditions were determined to be a laccase dosage of 40 U per gram absolute dry fiber (U/g), a treatment time of 120 min, a treatment temperature of 50 °C, and a proportion of BF of 20%. The mechanical properties of the binderless fiberboards prepared using a water bath were superior to spraying under the same conditions.

Keywords: Laccase; Self-bonding; Wheat straw fibers; Bamboo fibers; Mechanical properties; Spraying; Water bath; Fiberboards

*Contact information: Beijing Key Laboratory of Wood Science and Engineering, Beijing Forestry University, Beijing 100083, China; *Corresponding author: shuangbaozhangj5@163.com*

INTRODUCTION

Recently, concerns regarding the emission of formaldehyde from traditional petroleum-based adhesives in wood-based composites are growing; this has prompted board manufacturers to investigate environmentally-friendly composites to meet safer and healthier consumer demands. Binderless technology is potentially an effective solution to overcome formaldehyde emissions. In fact, systematic studies on binderless fiberboards began already in the 1980s. Binderless lignocellulose composites were prepared from bagasse (Mobarak *et al*. 1982); in subsequent years, processes were patented to produce binderless fiberboards based on bagasse, flax, and corn (Shen 1986). Thereafter, beech (Felby *et al.* 1997), spruce (Widsten *et al.* 2004), *Pinus radiata* (Riquelme-Valdes *et al.* 2008), and rubber wood fibers (Nasir *et al.* 2013) also exhibited potential use in binderless fiberboards.

Interest in manufacturing fiberboards using enzymatic treatments of cellulosic fibers has been growing in recent years (Kharazipour and Euring 2010; Euring *et al*. 2011a,b; Kudanga *et al*. 2011; Kharazipour and Euring 2013). Lignin-oxidising enzymes for bonding are based on the reactivity of phenoxy radicals in the plant cell wall. Using such methods involve no synthetic resin adhesives in the manufacturing process, and fiberboards are considered as green composites. In the last two decades, laccase because of its specific function of oxidizing lignin, mild reaction conditions, less by-product production, and its environmentally friendly behavior has received increased attention from researchers (Kharazipour *et al*. 1997; Felby *et al*. 2002; Felby *et al*. 2004; Widsten *et al*. 2004; Widsten and Kandelbauer 2008; Euring *et al*. 2013). Poplar binderless fiberboard has been fabricated using enzymatic methods, demonstrating that enzymatic treatment can improve the properties and performance of fiberboards (Zhou *et al*. 2013). Moreover, binderless fiberboards have been successfully produced with treated rubber wood fibers when the boards were prepared with 9.0 U/g of enzymes, 60 min treatment time, and pressed at 200 °C. Also, an excess amount of laccase, which consequently resulted in a decrease of internal bond strength (Nasir *et al*. 2013). During the treatment process, laccase primarily oxidizes lignin on the fiber surface without affecting other components (*e.g*. cellulose), with minimal penetration into the fiber (Álvarez *et al*. 2011; Moniruzzaman and Ono 2013). The treatment of fiber with laccase can activated the fiber surface by generating phenoxy radicals during oxidation process of phenolic hydroxyl groups of lignin. Phenolic groups are oxidized by laccase as follows (Felby *et al*. 2004; Widsten and Kandelbauer 2008; Álvarez *et al*. 2011):

$$4\mathrm{Phe}-\mathrm{OH}+\mathrm{O}_2 \xrightarrow{\mathrm{Laccase}} 4\mathrm{Phe}-\mathrm{O}^{\bullet}+2\mathrm{H}_2\mathrm{O} \tag{1}$$

Using laccase for bonding is based on the reactivity of phenoxy radicals in the plant cell wall. Then, laccase induced the generation of phenoxy radicals, which can generated covalent bonds and cross-linked by radical–radical coupling, and finally produce polymers (Ikeda *et al*. 1996; Felby *et al*. 2002). Therefore, self-bonding (fiber–fiber bonding) can be achieved, when fibers were treated with laccase.

New studies have attempted to use other raw materials than wood in order to prepare binderless fiberboards. Additionally, new forestry regulations, wood shortage, and the lower costs of non-woody raw materials have encouraged board producers to discover alternative wood sources (Halvarsson *et al*. 2009). Therefore, there has been increased interest in the use of agricultural by-products and residues (Álvarez *et al*. 2011). Compared to woody raw materials, the advantages of non-woody plants include cost-effectiveness and high availability in large quantities. The majority of non-woody straw remains underutilized and is eventually burned, resulting in pollution (Mo *et al*. 2001; Widsten and Kandelbauer 2008). Non-wood materials, including bagasse (Widyorini *et al*. 2005), coconut husk, and bamboo (Van Dam *et al*. 2004; Shao *et al*. 2009) have been evaluated for use as raw materials for producing binderless fiberboards.

Wheat straw, an agricultural residue of wheat production, is a potentially useful lignocellulosic material for producing binderless fiberboards. Non-resin wheat straw fiberboards have been manufactured from fibers that were pretreated with Fenton reagent (Halvarsson *et al*. 2009). The laccase pretreatment process is a reliable method for self-bonding technology (Nasir *et al*. 2015; Zhang et a*l*. 2015). Using laccase-treated wheat straw fibers in the production of binderless fiberboards has not yet been reported. Therefore, the purpose of this experiment is (i) to investigate the feasibility of binderless fiberboards from wheat straw fibers after laccase pretreatment, and (ii) to determine the effects of different laccase pretreatment conditions on the mechanical properties of binderless fiberboards. The ultimate goal was to determine the optimum conditions of laccase pretreatment for the preparation of high density binderless fiberboards from wheat straw fibers.

EXPERIMENTAL

Materials

Wheat straw fibers (WSF) were supplied by the Shandong Hailiyuan Green Packaging Materials Co., Ltd. (Shandong, China). WSF consisted of 70% wheat straw fibers (0.47 mm to 1.43 mm in length) and 30% wheat straw particles (1.0 mm to 8.5 mm in length), with a moisture content (MC) of 13%, and was used in this study. The major chemical composition of WSF was 40.3% cellulose, 24.6% hemicelluloses, and 21.0% lignin, which was provided by manufacturers. Bamboo fibers (BF) with a moisture content of 13 %, 0.51 mm to 1.16 mm in length, were used in this study. BF were provided by the Beijing Kenuo Senhua Wood Co., Ltd. (Beijing, China), and its major chemical composition was 35.2% cellulose, 26.5% hemicelluloses, and 27.6% lignin, which was provided by manufacturers. Liquid laccase, 1000 U/mL, was purchased from the Shanghai Danniyue Co., Ltd. (Shanghai, China). The laccase were generated from white rot fungi, and its suitable reactive conditions were a pH of 4.0 to 6.0, 25 °C to 75 °C treatment temperature, and 20 min to 240 min treatment time, which were provided by manufacturers.

Fiber Treatment

The WSF and BF were selected (target size < 6 mm), followed by screening with a twenty mesh screen, such that particles that passed through the screen were removed. The rest was rinsed with fresh water to remove soil. Afterwards, the WSF and BF were dried to a MC of 13% and mixed well. The mixed fibers (0.5 kg, a certain proportions of BF) were treated at 5% consistency in aqueous suspension under different parameters, such as variation of laccase dosages (20.0, 40.0, and 60.0 U per gram absolute dry fiber (U/g)), variation of treatment time (60, 120, and 180 min), variation of treatment temperature (30, 50, and 70 °C), variation of proportions of BF (0, 10, and 20 %). These parameters are shown in Table 1. Sodium acetate and acetic acid were used as a buffering solution, and pH adjustment was made to maintain 4.5. In order to obtain a homogeneous suspension and ensure a sufficient supply of oxygen for the enzyme reaction, the suspension was stirred and provided with air supply until treatment achieved. After treatment, the fibers were washed to reach neutral pH, and they were filtered and dried to a MC of approximately 13%. The dried material was used for producing binderless fiberboards. The addition of laccase included two different techniques: water bath (Method 1) and spraying (Method 2). For Method 2, laccase was added by spray propulsion during the mixing process.

Manufacturing Process of Binderless Fiberboards

Binderless fiberboards were prepared from the laccase-treated fibers with a target density of 1000 kg/m^3 and a target thickness of 5 mm, which was controlled by a thickness gauge. The mixed fibers (0.50 kg, a MC of 13%) were formed to a mat with a forming box (300 mm in length ×300 mm in width). Then, the mat was loaded into hot pressing. The boards were manufactured at 170 °C under 4 MPa for 15 min. The obtained fiberboards are referred herein as I and II, depending on Method 1, Method 2 resp., and boards were manufactured under the same conditions, with the exception of the mode of application of the laccase. Additionally, additional experiments were conducted and additional boards were pressed under optimized parameters.

Evaluation of Mechanical and Physical Properties

The mechanical properties including the modulus of rupture (MOR), modulus of elasticity (MOE), internal bond strength (IB), and thickness swelling (TS) after (20±2) °C water immersion for 2 h, were evaluated. Before the evaluation of mechanical and physical properties, the boards were conditioned at 25 °C and 65% relative humidity until mass equilibrium. For each group, three boards were prepared, and six samples were tested for MOR, MOE, IB, and 2h TS, a total of 24 samples. The tests were conducted according to the Chinese Industrial Standard, GB/T21723-2008 (2008).

Experiment Design

The experiment was designed as a series of single factor experiments. As shown in Table 1, there were four factors and each factor had three levels. This study was designed to investigate one factor at a time while keeping the rest of the factors constant. As an example, the effect of laccase dosage on the mechanical properties of the fiberboards was investigated using the following experimental conditions: 120 min treatment time, 70 °C treatment temperature, 10 % bamboo fibers and variation of the laccase dosage: 20 U/g, 40 U/g, and 60 U/g resp.; by this approach the optimal laccase dosage was finally determined. The influence of the other parameters was determined in a similar way, always keeping the rest factors constant.

Table 1. Single-Factor Experimental Design

Group	Laccase Dosage (U/g)		Treatment Time (min)		Treatment Temperature (°C)		Proportions of BF (%)	
	I	II	I	II	I	II	I	II
1	20		120		70		10	
2	40		120		70		10	
3	60		120		70		10	
4	40		60		70		10	
5	40		120		70		10	
6	40		180		70		10	
7	40		120		30		10	
8	40		120		50		10	
9	40		120		70		10	
10	40		120		70		0	
11	40		120		70		10	
12	40		120		70		20	
Note: Ⅰ-Method 1, Ⅱ-Method 2, U/g-U per gram absolute dry fiber, BF-bamboo fibers.								

RESULTS AND DISCUSSION

The mechanical properties of binderless fiberboards produced with laccase-treated fibers were evaluated by MOR, MOE, and IB, and the results are given in Table 2. To elucidate the effects of each factor on the mechanical properties of binderless fiberboards, Figs. 1 to 4 are used to further describe for the differences in different levels of each factor.

Table 2. Results of Single-Factor Experiment

Group	**MOR(MPa)**		**MOE(MPa)**		**IB(MPa)**	
	I	**II**	**I**	**II**	**I**	**II**
1	10.130 (0.813)	11.147 (0.924)	1178.870 (146.802)	1226.257 (124.977)	0.110 (0.014)	0.097 (0.009)
2	19.627 (0.822)	19.503 (1.208)	2849.377 (217.439)	2744.687 (242.988)	0.243 (0.025)	0.220 (0.022)
3	14.430 (1.011)	13.687 (1.654)	2442.887 (241.270)	2384.357 (251.270)	0.240 (0.024)	0.233 (0.021)
4	14.397 (1.161)	13.733 (1.507)	2076.197 (190.641)	1981.797 (213.117)	0.187 (0.021)	0.173 (0.017)
5	18.650 (1.909)	19.390 (2.430)	2775.913 (252.547)	2796.313 (203.027)	0.237 (0.021)	0.230 (0.022)
6	16.780 (1.061)	17.340 (0.984)	2557.503 (219.950)	2418.007 (185.041)	0.247 (0.029)	0.233 (0.025)
7	13.050 (1.109)	13.590 (1.343)	1982.847 (200.128)	1917.053 (84.941)	0.167 (0.012)	0.157 (0.017)
8	20.750 (1.444)	20.153 (1.469)	2944.310 (137.949)	2905.843 (100.605)	0.253 (0.017)	0.243 (0.031)
9	19.497 (1.978)	18.960 (0.861)	2806.777 (144.743)	2679.137 (241.789)	0.240 (0.016)	0.237 (0.012)
10	20.360 (1.613)	20.953 (1.910)	2941.827 (180.850)	2830.007 (195.242)	0.220 (0.022)	0.223 (0.021)
11	18.730 (1.741)	18.930 (1.800)	2826.173 (244.153)	2756.107 (223.601)	0.237 (0.029)	0.233 (0.025)
12	18.113 (1.925)	17.647 (1.564)	2544.203 (236.794)	2496.810 (276.289)	0.253 (0.026)	0.247 (0.024)

Note: Ⅰ-Method 1, Ⅱ-Method 2, MOR-Modulus of rupture, MOE-Modulus of elasticity, IB-Internal bond strength. The value in brackets is the standard deviation.

Using laccase for bonding is based on the reactivity of phenoxy radicals in the plant cell wall. To some degree, the dosage of laccase determines the amount of generated phenoxy radicals (Felby *et al*. 2002; Müller *et al*. 2009). Figure 1 reveals the effect of the laccase dosage on the mechanical properties of binderless fiberboards with laccase-treated WSF.

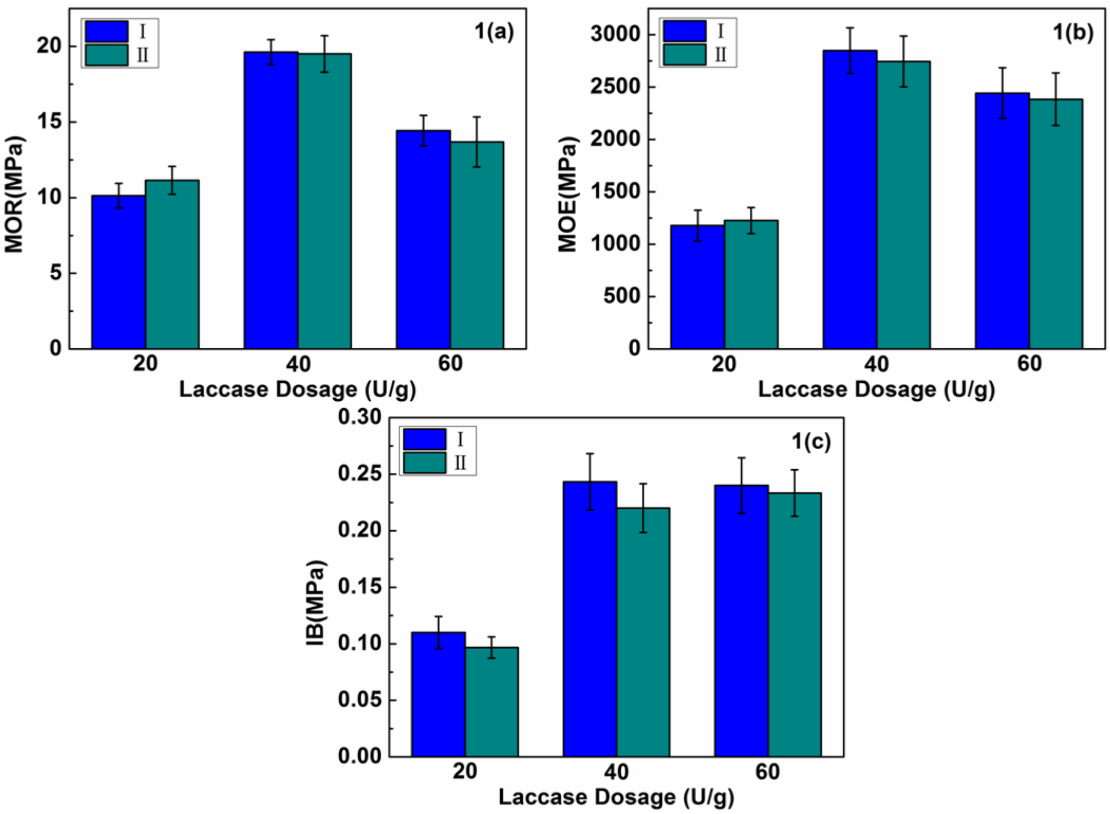

Fig. 1. Effect of laccase dosage on the mechanical properties of binderless fiberboards

As can be seen, 20 U/g was not enough to generate a huge amount of radicals and obtain better mechanical properties, thereby exhibiting lower mechanical properties. As the laccase dosage increased from 20 U/g to 40 U/g, MOR, MOE, and IB exhibited a notable increase. Reasons for the improvement of IB are that laccase activated the fiber surface and then generated more free radicals when the fibers were treated with the laccase. Free radicals could act on sites for further cross-linking reactions. Simultaneously, as the reaction proceeds, the activated lignin molecules reacted among each other and became partially repolymerized (Müller *et al*. 2009). Moreover, the surface of fibers generated a thin layer of depolymerized lignin depositions, which were helpful for the fiber bonding during the hot-pressing process (Maximova *et al*. 2001; Felby *et al*. 2002; Koljonen *et al*. 2004; Nasir *et al*. 2013). Thereby, the highest IB was obtained under a laccase dosage of 40 U/g. On the other hand, because of the removal of extracellular, amorphous lignin bonded loosely to the cellulose fiber, laccase can increase the crystallinity of fibers (Nasir *et al*. 2013), which led to the improvement of MOR and MOE.

Although higher laccase dosages result in better bonding and improving crystallinity of fibers, excessive laccase can significantly decrease crystallinity of the cellulose component because degradation of the intracellular lignin and phenolic compounds decreased the crystallinity of cellulose component (Nasir *et al*. 2013). However, with the increase of laccase dosage, deposited lignin continuously increased, causing the decline of the content of lignin. By this reason the IB of boards did not exhibit any remarkable increase, whereas MOR and MOE exhibited a dramatic decrease because of the decrease of crystallinity. This phenomenon can be seen in Fig. 1. The maximum value of MOR, MOE, and IB could be observed when the laccase dosage was 40 U/g, and a laccase dosage of 40 U/g can be advised on the basis of this study.

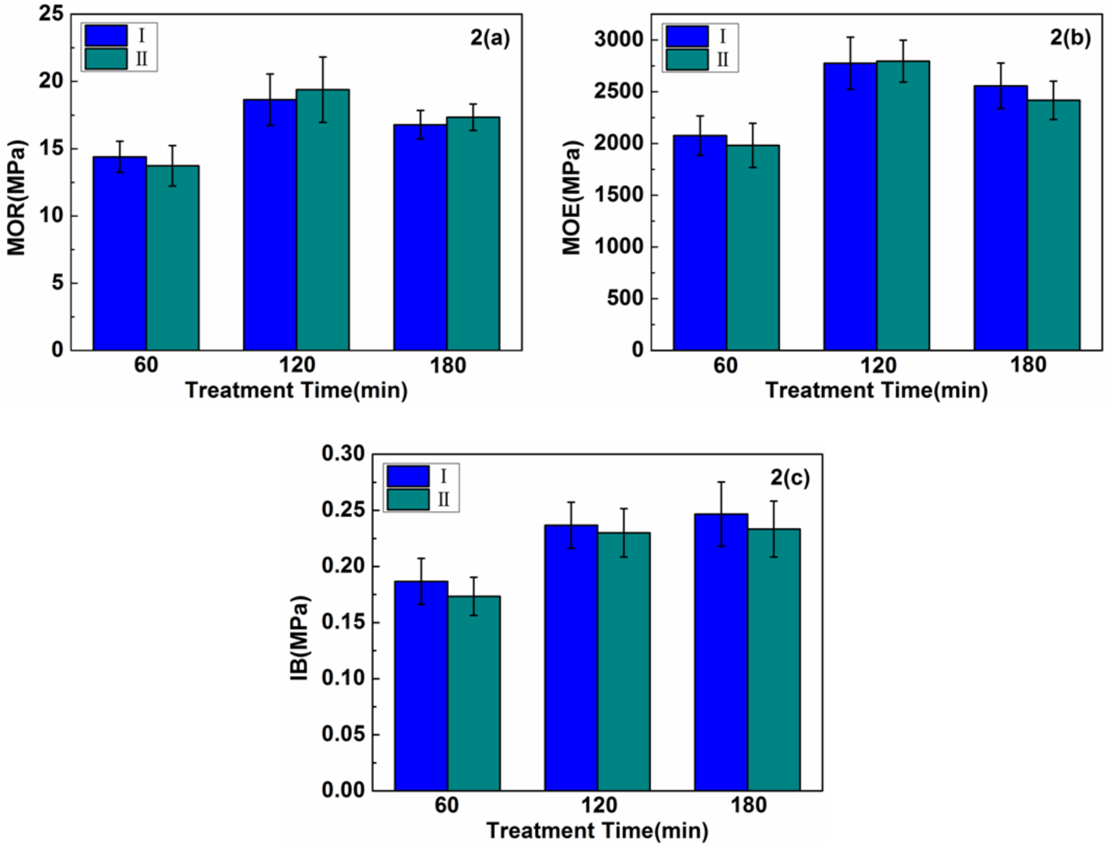

Fig. 2. Effect of treatment time on the mechanical properties of binderless fiberboards

The effect of treatment time on the mechanical properties could be achieved by promoting or restricting the enzyme reaction. The reactions between laccase and lignin on the fiber surface were accomplished completely only in the first 2 h after incubation started because radicals remained the same or did not exhibit an obvious increase after two hours (Müller *et al*. 2009). Therefore, three different treatment times: 60 min, 120 min, and 180 min, resp., were selected. Figure 2 shows the effect of the treatment time on the mechanical properties of binderless fiberboards with laccase-treated WSF.

Lower mechanical properties were obtained under conditions of 60 min. This may be attributed to the fact that enzyme reactions could not be achieved completely. By increasing the treatment time, an additional increase of the mechanical properties could be observed because enough time can make sure that enzyme reaction is accomplished completely. The best mechanical properties were exhibited under conditions of 120 min. This result could be ascribed to the fact that a certain increase of treatment time contributed to the increasing of crystallinity and better bonding of fibers. However, extended treatment time could lead to a slight decrease of fiber crystallinity, and finally causing the decline of MOR and MOE. However, the decline was not obvious compared to the effect of excessive laccase on MOR and MOE. The IB of boards remained slightly increased because longer treatment time could generate more lignin deposition, and the presence of lignin depositions was beneficial for bonding of fiber-fiber. It is obvious that too long treatment times did not improve board properties. Based on this study, a treatment time of 120 min was advised in order to obtain better mechanical properties.

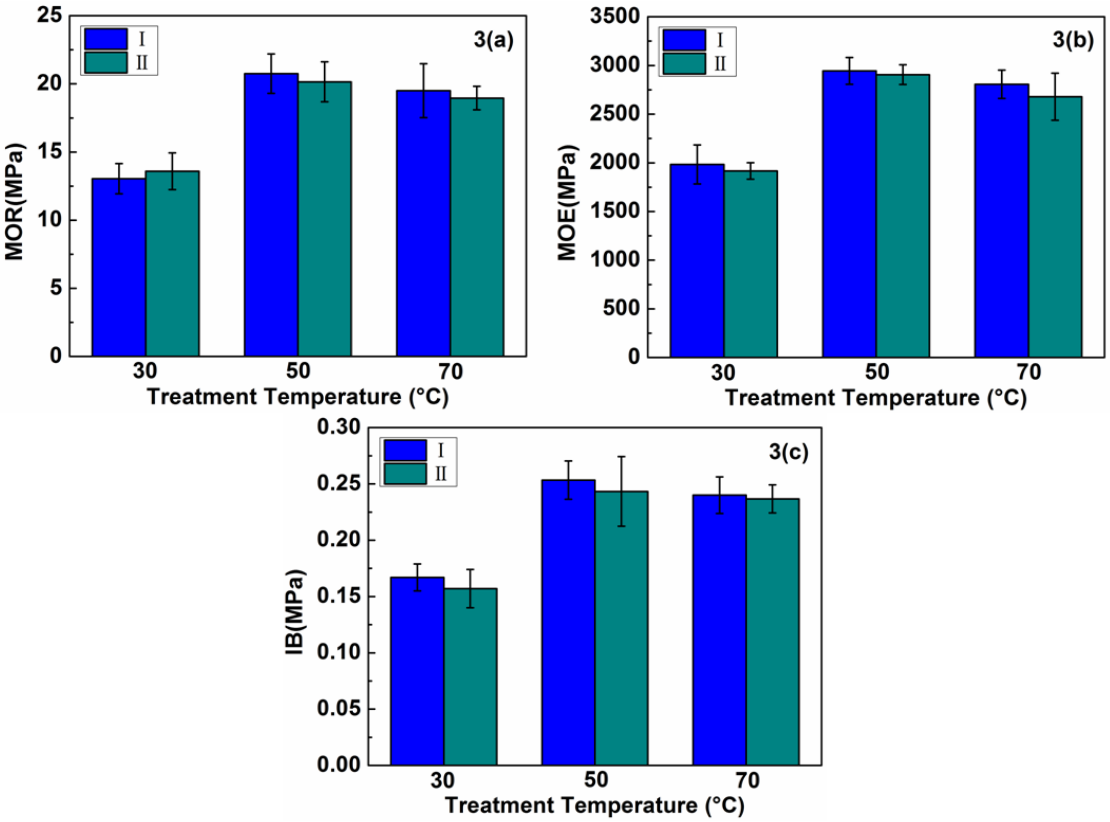

Fig. 3. Effect of treatment temperature on the mechanical properties of laccase-treated binderless fiberboards

The effect of treatment temperature on the mechanical properties could be achieved by promoting or restricting the enzyme reaction. The concentration of reactive oxygen species (ROS) free radicals was influenced by treatment temperature, and the highest values of ROS concentrations can achieved at a certain treatment temperature (Cao *et al*. 2008). Figure 3 shows the effect of the treatment temperature on the mechanical properties of binderless fiberboards with laccase-treated WSF.

Laccase belongs to the substance class of protein, for which a higher or lower temperature can restrict and even lose its catalytic ability. Laccase is able to play its catalytic ability within a suitable range of treatment temperature. Under the conditions of 30 °C, enzyme reactions were significantly restricted, and laccase could not play its catalytic ability, showing worse activated degree in the fiber surface. Compared to 30 °C, the restriction was not more evident when the treatment temperature was 70 °C. It may be inferred that 70 °C is closer to the optimal treatment temperature. From the Fig 3, in this study, it may be concluded that 50 °C was a better treatment temperature for enzymatic reactions than 30 °C and 70 °C, hence improving the mechanical properties.

The characteristics of the raw materials were a major factor affecting mechanical properties. Figure 4 shows the effect of the proportion of BF on the mechanical properties of laccase-treated WSF binderless fiberboards.

MOE and MOR exhibited a clear decreasing trend in the range up to 20%, which mostly was due to the characteristics of the raw materials. The length of the BF was shorter than that of the WSF, which fact resulted in the reduction of MOE and MOR. In contrast, the proportion of BF had a considerable positive effect on IB.

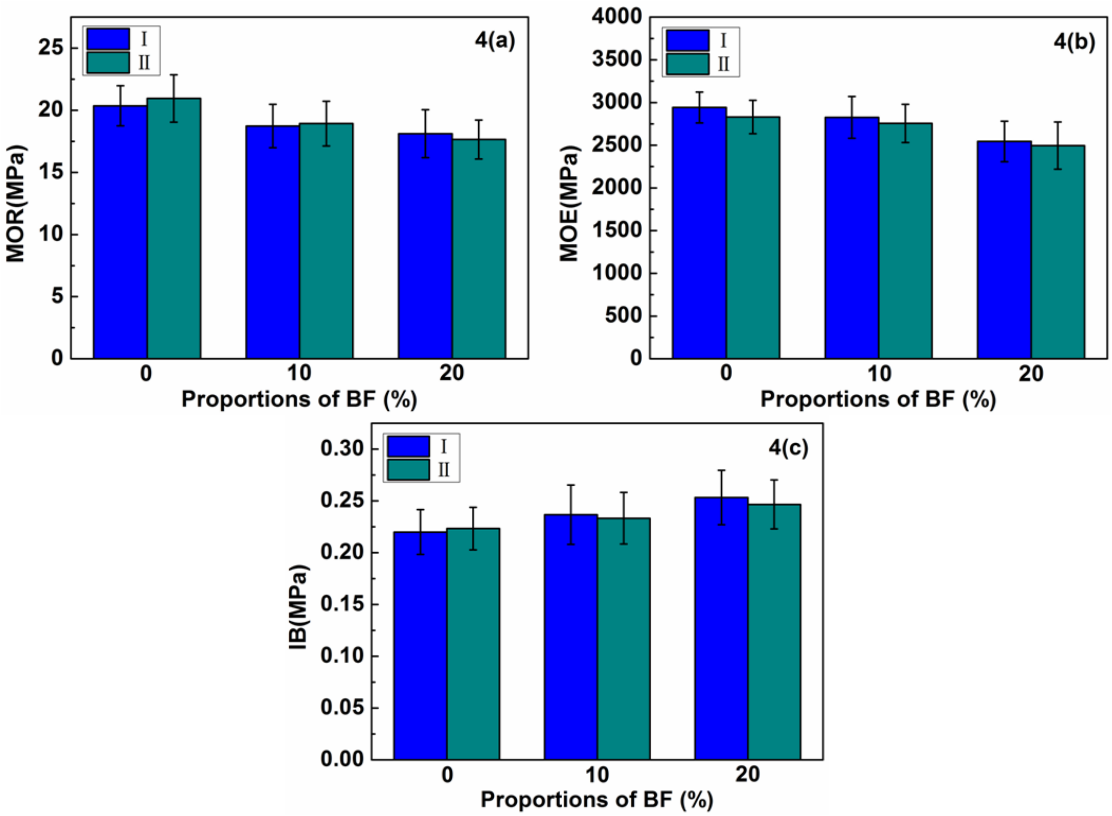

Fig. 4. Effect of proportions of bamboo fiber (BF) on the mechanical properties of laccase-treated binderless fiberboards

There are two possible explanations for this result: (1) BF has a higher content of lignin (mentioned in the section on Materials) and therefore, the enzymatic oxidation could generate more phenol hydroxyl radicals available to react, and hence this characteristic made it possess better self-bonding strength; or (2) The addition of BF improved the degree of silicon dispersion of WSFs, thereby improving the bonding condition; this led to an improvement in IB. When the proportion of BF was 20%, the value of IB achieved the maximum value, meaning that this condition was optimal. As the proportion of BF continuously increased to 30%, the IB of boards remained slightly increased, whereas MOR and MOE exhibited a notable decrease. 30% BF was not considered in this study. Therefore, the addition 20% BF can be recommended.

Based on the above results, the optimal parameters for preparing binderless fiberboards with laccase-treated WSF within the experimental frame of the work described in this paper were 40 U/g, 120 min, 50 °C, and 20 % BF. Additionally, the effects among different laccase dosages, treatment times, treatment temperatures, and proportions of BF on the mechanical properties were in most cases similar for Method 1 (water bath) and Method 2 (spraying). For MOE and MOR two methods showed rather the same results; for IB, method 1 gave better results in most cases. It can be concluded that the laccase dosage and the proportions of BF can be considered as the main parameters affecting the mechanical properties of WSF binderless fiberboards. The effects of treatment time and treatment temperature on the mechanical properties were presented and achieved by influencing the enzyme reaction.

The values of TS, over 30%, generally were rather high, and they exceeded the requirements (≤ 6.0 %). This result was mostly due to the absence of water-repellent agent such as added paraffin. Another reasonable explanation was that the self-bonded fibers were easily dispersed because of the nature of WSF with their inherent high content of SiO_2 when boards were immersed in water. Moreover, lower molar mass carbohydrates produced by hydrolysis of cellulose and hemicelluloses under high temperature and moderate moisture could have a certain positive effect on TS, as a consequence, increasing TS. Therefore, 2 h TS, without showing some dependencies with the discussed factors, is not discussed here. Moreover, another common drawback of obtained boards is a lower IB due to the nature of WSF with their inherent high content of SiO_2 hindering the formation of good bond strength between fibers.

Table 3. Results of Optimization Experiments

MOR(MPa)		MOE(MPa)		IB(MPa)	
I	**II**	**I**	**II**	**I**	**II**
18.877 (1.491)	18.223 (1.582)	2743.830 (225.574)	2631.190 (199.660)	0.273 (0.025)	0.260 (0.022)

Note: Ⅰ-Method 1, Ⅱ-Method 2, MOR-Modulus of rupture, MOE-Modulus of elasticity, IB-Internal bond strength. The value in brackets is the standard deviation.

Additional experiments were conducted under optimized parameters, 40 U/g, 120 min, 50 °C, and 20 % BF. Table 3 shows the results for the mechanical properties of binderless fiberboards with laccase-treated WSF and BF. Under optimal conditions, boards were successfully produced by dry forming at 170 °C at 4 MPa for 15 min, showing satisfactory mechanical properties. The mechanical properties exhibited a certain improvement, especially the IB; the MOR and MOE of the boards met the requirements of GB/T21723 (2008) (15 MPa and 1950 MPa). Unfortunately, all of the samples showed some inadequacies in IB and the 2 h TS, which did not meet the testing standards of IB $\geq$ 0.45 Mpa and 2 h TS $\leq$ 6.0%. Therefore, further optimization is necessary to improve IB and 2 h TS. Boards manufactured with laccase treatment combined with mediators may be likely to improve the IB. Additionally, the addition of a waterproofing agent might decrease the 2 h TS to meet the requirements.

CONCLUSIONS

1. This study showed that laccase-treated WSF can be used to produce binderless fiberboards with good mechanical properties. However it must be considered that the press time was extremely long. Whether or not shorter press times will give the same results needs further evaluation.The effects of laccase dosage, treatment time, treatment temperature, and the proportion of BF on the mechanical properties of the binderless fiberboard of Methods 1 and 2 exhibited a similar trend; however, the procedure using the water bath was superior compared to spraying under the same processing conditions.
2. The optimal conditions for the preparation of binderless fiberboards with laccase-treated WSF and BF were determined to be a laccase dosage of 40 U/g, a treatment time of 120 min, a treatment temperature of 50 °C, and a proportion of BF of 20 %.

Additionally, the laccase dosage and the proportions of BF were the main parameters affecting the mechanical properties binderless fiberboards. Based on optimal parameters, the MOR and MOE met the standard requirements; whereas all of the samples exhibited some inadequacies in IB and 2 h TS. Therefore, further optimization is necessary to conduct.

ACKNOWLEDGMENTS

This research is supported by the Co-Built Foundation with Beijing Municipal Education Commission R&D on Key Technology in Scientific Utilization of Non-Wood Plant Raw Materials, the Beijing Natural Science Foundation (6162019), and the Co-Built Foundation with Zhejiang Province (CZXC201410) R&D on Bamboo Composites and Environmentally Friendly Adhesives. The authors are additionally grateful for support from the Zhejiang Chengzhu Advanced Material Technology Co., Ltd. and the Anhui Sentai WPC Group Co., Ltd.

REFERENCES CITED

Álvarez, C., Rojano, B., Almaza, O., Rojas, O. J., and Gañán, P. (2011). "Self-bonding boards from plantain fiber bundles after enzymatic treatment: Adhesion improvement of lignocellulosic products by enzymatic pre-treatment," *Journal of Polymer and the Environment* 19(1), 182-188. DOI: 10.1007/s10924-010-0260-6

Cao, Y. J., Duan, X. F., Cao, Y. L., Lü, J. X., Zhu, J. Q., Zhou, G. W., and Zhao, B. L. (2008). "ESR study in reactive oxygen species free radical production of *Pinus kesiya*, var. langbianensis, heartwood treated with laccase," *Applied Magnetic Resonance* 35(1), 205-211. DOI: 10.1007/s00723-008-0149-x

Euring, M., Ruhl, M., Ritter, N., Kües, U., and Kharazipour, A. (2011a). "Laccase mediator systems for eco-friendly production of medium-density fiberboard (MDF) on a pilot scale: Physicochemical analysis of the reaction mechanism," *Biotechnology Journal* 6(10), 1253-1261. DOI: 10.1002/biot.201100119

Euring, M., Trojanowski, J., Horstmann, M., and Kharazipour, A. (2011b). "Studies of enzymatic oxidation of TMP-fibers and lignin model compounds by a laccase-mediator-system using different ^{14}C and ^{13}C techniques," *Wood Science and Technology* 46(4), 699-708. DOI: 10.1007/s00226-011-0439-6

Euring, M., Trojanowski, J., and Kharazipour, A. (2013). "Laccase-mediator catalyzed modification of wood fibers: Studies on the reaction mechanism and making of medium-density fiberboard," *Forest Production Journal* 63(1-2), 54-60. DOI: 10.13073/FPJ-D-12-00075

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-catalyzed oxidation of wood fibers from beech (*Fagus sylvatica*)," *Applied Microbiology and Biotechnology* 48(4), 459-464. DOI: 10.1007/s002530051080

Felby, C., Hassigboe, J. and Lund, M. (2002). "Pilot-scale production of fiberboard´s made by laccase oxidized wood fibers: Board properties and evidence for cross-linking of lignin," *Enzyme & Microbial Technology* 31(6), 736-741. DOI: 10.1016/S0141-0229(02)00111-4

Felby, C., Thygesen, L. G., Sanadi, A., and Barsberg, S. (2004). "Native lignin for bonding of fiberboards-evaluation of bonding mechanisms in boards made from laccase-treated fibers of beech (*Fagus sylvatica*)," *Industrial Crop and Products* 20(2), 181-189. DOI: 10.1016/j.indcrop.2004.04.020

GB T21723. (2008). "Wheat/rice-straw particleboard," *Standardization Administration of China*, Beijing, China.

Ikeda, R., Uyama. V., and Kobayashi, V. (1996). "Novel synthetic pathway to a poly (phenylene oxide). Laccase-catalyzed oxidative polymerization of syringic acid," *Macromolecules* 29(8), 3053-3504. DOI: 10.1021/ma951810b

Halvarsson, S., Edlund, H., and Norgren, M. (2009). "Manufacture of non-resin wheat straw fiberboards," *Industrial Crops and Products* 29(2), 437-445. DOI: 10.1016/j.indcrop.2008.08.007

Kharazipour, A., Hüttermann, A. and Lüdemann, H.D. (1997). "Enzymatic activation of wood fibers as a mean for the production of wood composites," *Journal of Adhesion Science and Technology* 11(3), 419-427. DOI: 10.1163/156856197X00796

Kharazipour, A., and Euring, M. (2010). "Use of mediators in the production of fibreboards," U.S. Patent, WO 2010020409 A3

Kharazipour, A., and Euring, M. (2013). "Method for producing wood materials and/or composite materials," U.S. Patent, WO 2013127947 A1

Koljonen, K., Österberg, M., Kleen, M., Fuhrmann, A., and Stenius, P. (2004). "Precipitation of lignin and extractives on kraft pulp: Effect on surface chemistry, surface morphology and paper strength," *Cellulose* 11(2), 209-224. DOI: 10.1023/B:CELL.0000025424.90845.c3

Kudanga, T., Nyanhongo, G. S., Guebitz, G. M., and Burton, S. (2011). "Potential applications of laccase-mediated coupling and grafting reactions: A review," *Enzyme and Microbial Technology* 48(3), 195-208. DOI: 10.1016/j.enzmictec.2010.11.007

Mobarak, F., Fahmy, Y., and Augustin, H. (1982). "Binderless lignocellulose composite from bagasse and mechanism of self-bonding," *Holzforschung* 36(3), 131-136. DOI: 10.1515/hfsg.1982.36.3.13

Maximova, N., Österberg, M., Koljonen, K., and Stenius, P. (2001). "Lignin adsorption on cellulose fibre surfaces: Effect on surface chemistry, surface morphology and paper strength," *Cellulose* 8(2), 113-125. DOI: 10.2013/A:1016721822763

Mo, X., Hu, J., Sun, X. S., and Ratto, J. A. (2001). "Compression and tensile strength of low-density straw-protein particleboard," *Industrial Crop and Products* 14(1), 1-9. DOI: 10.1016/S0926-6690(00)00083-2

Moniruzzaman, M., and Ono, T. (2013). "Separation and characterization of cellulose fibers from cypress wood treated with ionic liquid prior to laccase treatment." *Bioresource technology* 127(1), 132. .DOI: 10.1016/j.biortech.2012.09.113

Müller, C., Euring, M. A. and Kharazipour, A. (2009). "Enzymatic modification of wood fibres for activating their ability of self bonding." *International Journal of Materials and Product Technology* 36(1-4), 189-199. DOI: 10.1504/IJMPT.2009.02783

Nasir, M., Gupta, A., Beg, M., Chua, G. K., Jawaid, M., Kumar, A., and Khan, T. A. (2013). "Fabricating eco-friendly binderless fiberboard from laccase-treated rubber wood fiber," *BioResources* 8(3), 3599-3608. DOI: 10.15376/biores.8.3.3599-3608

Nasir, M., Hashim, R., Sulaiman, O., Nordin, N. A., Lamaming, J., and Asim, M. (2015). "Laccase, an emerging tool to fabricate green composites: A review." *BioResources* 10(3), 6262-6284. DOI: 10.15376/biores.10.3.Nasir

Riquelme-Valdes, J., Ramirez, E., Contreras, D., Freer, J., and Rodriguez, J. (2008). "Fiberboard manufactured without resin using the Fenton reaction," *Journal of Chilean Chemical Society* 53(4), 1722-1725. DOI: 10.4067/S0717-97072008000400019

Shao, S., Jin, Z., Wen, G., and Iiyama, K. (2009). "Thermo characteristics of steam-exploded bamboo (*Phyllostachys pubescens*) lignin," *Wood Science and Technology* 43(7-8), 643-652. DOI: 10.1007/s00226-009-0252-7

Shen, K. C. (1986). "Process for manufacturing composite products from lignocellulosic materials," US Patents 4,627,951.

Van Dam, J. E., van den Oever M. J., Teunissen, W., Keijsers, E. R., and Peralta, A. G. (2004). "Process for production of high density/high performance binderless boards from whole coconut husk. Part 1: Lignin as intrinsic thermosetting binder resin," *Industrial Crops and Products* 19(3), 207-216. DOI: 10.1016/j.indcrop.2003.10.003

Widsten, P., Tuominen, S., Qvintus-Leino, P., and Laine, J.E. (2004). "The influence of high defibration temperature on the properties of medium-density fiberboard (MDF) made from laccase-treated softwood fibers," *Wood Science and Technology* 38(7), 521-528. DOI: 10.1007/s00226-003-0206-4

Widsten, P., and Kandelbauer, A. (2008). "Adhesion improvement of lignocellulosic products by enzymatic pre-treatment," *Biotechnology Advances* 26(4), 379-386. DOI: 10.1016/j.biotechadv.2008.04.003

Widyorini, R., Higashihara, T., Xu, J., Watanabe, T., and Kawai, S. (2005). "Self-bonding characteristics of binderless kenaf core composites," *Wood Science and Technology* 39(8), 651-662. DOI: 10.1007/s00226-005-0030-0

Zhang, D., Zhang, A., and Xue, L. (2015). "A review of preparation of binderless fiberboards and its self-bonding mechanism," *Wood Science and Technology* 49(4), 661-679. DOI 10.1007/s00226-015-0728-6

Zhou, X., Zheng, F., Lv, C., Tang, L., Wei, K., Liu, X., Du, G., Yong, Q., and Xue, G. (2013). "Properties of formaldehyde-free environmentally friendly lignocellulosic composites made from poplar fibres and oxygen-plasma-treated enzymatic hydrolysis lignin," *Composites Part B: Engineering* 53, 369-375. DOI: 10.1016/j.compositesb.2013.05.037

Article submitted: July 8, 2016; Peer review completed: August 14, 2016; Revised version received: March 22, 2017; Accepted: March 28, 2017; Published: April 3, 2017, DOI: 10.15376/biores.12.2.3707-3719