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ORIGINAL ARTICLE

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Use of hemicellulase in sequence with hydrogen peroxide and laccase for improvement of teak veneer surface color

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Abstract Teak veneer was treated with a commercial hemicellulase from *Thermomyces lanuginosus* in sequence with hydrogen peroxide or a hydrogen peroxide-laccase mixture to improve the veneer surface color and at the same time to reduce the necessary amount of hydrogen peroxide. The removal of a small portion of hemicellulose from the teak veneer surface could be carried out after treatment with the hemicellulase preparation as 0.05 xylanase unit/ml for 30 min. The veneer samples immediately after the hemicellulase treatment were subjected to the designated concentration of hydrogen peroxide or hydrogen peroxide-laccase mixture at 60°C at pH 6.5 for 4 h. The changes in veneer color were measured by using imaging technology as percentage change in gray scale. A treatment combination of hemicellulase containing xylanase, hydrogen peroxide, and laccase yielded a color improvement close to that achieved using 20% hydrogen peroxide. The results showed that hemicellulase pretreatment could improve the bleachability of teak veneer surfaces treated with hydrogen peroxide or hydrogen peroxide-laccase mixture.

Key words Teak veneer · Hemicellulase · Laccase · Hydrogen peroxide · Color improvement

Introduction

Teak (*Tectona grandis*), a hardwood, grows in the northern part of Thailand and is known for its beautiful color and texture as well as its resistance to termites. To conserve this precious wood, plywood using teak as a face veneer[1] has generally replaced solid teak wood. The use of wood in this manner also adds value to the cheaper and lower quality inner and back veneer wood, which otherwise cannot be used as decorative plywood. Nowadays, even with the use of veneer in place of solid teak wood, shortage in the supply of teak continues. Therefore, commercially grown teaks have been developed to help replenish the demand for natural teak. As a result of fertilization and cultivation, however, many farmed teaks have a dark color and dark prominent growth rings, which are different from the golden-yellowish color of the naturally grown teak, and thus are considered low quality and undesirable. The lack of good color quality in commercially grown teak has created a demand for color improvement.

To our knowledge, no findings have ever been published regarding teak veneer color improvement. Therefore, decolorizing materials in this research were selected from those that are normally used in decolorization processes such as pulp and paper bleaching, wastewater decolorizing, and textile bleaching. Care had to be taken not to destroy the veneer texture. Based on existing decolorizing methods, the best way to lighten the color of wood and pulp is to use chlorine, chlorine dioxide, or chemicals containing chlorine because of their powerful bleaching capability. Because of their negative impact on the environment, these chemicals are being replaced by other less harmful materials.[2,3] Therefore, the combination of H_2O_2, laccase, and hemicellulase containing xylanase from *Thermomyces lanuginosus* were selected to improve teak veneer color.

Teak is composed primarily of cellulose, hemicelluloses, and lignin. The double bonds responsible for wood color are absent in cellulose and hemicellulose but present in lignin. The lignin is considered darker in nature.[4] An informative and extensive review on lignin has been published by Higuchi.[5]

The hypothesis of this research is to hydrolyze a small portion of hemicellulose that is bonded together with lignin. Loosening some hemicellulose-lignin bonds through enzymatic scission of hemicellulose might provide easier access of laccase and H_2O_2 to lignin and improve the bleachability of the teak veneer surface.[2,6–9] Viikari et al.[10] used xylanase to treat kraft pulp and found that xylanase decreased the amount of chemical used.

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Digital imaging technology was adopted to measure the improvement of the teak veneer surface color. This technology was used instead of lignin analysis because of its rapid measurement and lack of chemical involvement. The image and shading level of teak veneer samples were taken before and after the color improvement treatment and then converted into numerical values. The differences in numerical values were used to determine the level of changes in color improvement.

The objective of this research is to improve the color quality of teak veneer surfaces using a small amount of hemicellulase in sequence with H_2O_2 or H_2O_2-laccase mixture treatment. The goal is to use environmentally friendly agents, minimizing their amounts.

Materials and methods

Materials

Commercial hemicellulase containing xylanase (EC 3.2.1.8) from *Thermomyces lanuginosus*, laccase (EC 1.10.3.2) from *Rhus vernicifera*, xylan from oat spelts, and syringaldazine were purchased from Sigma. Dibasic sodium phosphate heptahydrate and xylose were purchased from Fluka. The rest of the chemicals were purchased from Merck.

The teak veneer samples, provided by Metro Top Wood Co., Ltd., Thailand, were commercially grown teaks from northern Thailand. The samples were randomly selected from teak with color quality problem and with the following qualifications: yellow/brown color, 0.05 cm in thickness, no prior bleaching, no scarring, and no reaction wood.

Sample preparation

Each piece of veneer was cleaned with a damp cloth and carefully cut into square pieces of 3.2×3.2 cm, and then left to dry for 1 day at 25°C. The samples were then stored in a labeled Ziplock plastic bag until used.

Experimental procedure

The experiments were designed to study the effect of hemicellulase, H_2O_2, and laccase concentration. For all experiments, the veneer samples were placed into 150-ml beakers containing 20 ml of the designated solution and covered with plastic wrap to ensure a closed system. The temperature of the system was controlled by placing the beaker in a temperature-controlled water bath shaker (TAITEC xy-80). At the scheduled time, the veneer samples were removed from the solutions, washed with water, dried with a cloth, and left to dry for 1 day in the 25°C temperature-controlled room. The samples were collected and scanned using the procedure described in the image analysis section. Data presented were the average of three replicates ± the standard error of the mean.

H_2O_2 treatments

The treatments of H_2O_2 solely on teak veneer surfaces at different concentrations (5%, 10%, 15%, and 20%) and different reaction times (0.5, 1, 2, and 4 h) at 60°C and pH 6.5 were carried out to compare color improvement with the proposed treatment in this research.

Teak veneer hemicellulose hydrolysis

Teak veneer hemicellulose hydrolysis experiments were performed at different hemicellulase concentrations [0.05, 0.50, 1.00, and 2.50 xylanase unit/milliliter (U/ml)] and reaction times (0.25, 0.5, 1, 2, 4, and 24 h) at 32°C and pH 4.5, to acquire the adequate amount of hemicellulase and reaction time.

The sample solutions were measured for the reducing sugars (the hydrolysis products) concentration using a UV-2450 UV-visible spectrophotometer (Shimadzu). The veneer hemicellulose hydrolysis rates in micrograms of xylose equivalents per hour per unit area of the veneer samples ($\mu g/h\text{-}cm^2$) were also calculated to compare and determine suitable conditions. Furthermore, the solution samples obtained from veneer treatments were also analyzed for sugar composition using high performance liquid chromatography (HPLC) from Alltech with an Amino (5 μm) HPLC-Cartridge 250–4 Lichrospher 100 column. The mobile phase was 85% volume by volume acetonitrile in water at 1.5 ml/min flow rate. The detector was an Evaporative Light Scattering Detector (ELSD) model 2000ES.

H_2O_2 and laccase treatments

In the second treatment, after the hemicellulase pretreatment, the effect of H_2O_2 alone at different concentrations (5% and 10%) and combination of different H_2O_2–laccase mixture concentrations (0, 0.01, 0.03, 0.05, 0.25, and 1 U/ml) on color reduction was investigated. The experimental conditions were fixed at 60°C, pH 6.5 (regulated by 0.02 M phosphate buffer) and 4 h reaction time.

Enzyme assays

Commercial hemicellulase was assayed for xylanase activity by measuring the reducing sugar released from hydrolysis of oat spelts xylan using the Nelson-Somogyi method[11] with xylose as the standard sugar. One unit was defined as the amount of enzyme required to liberate 1 micromole of reducing sugar as xylose equivalents from xylan per minute at pH 4.5 (regulated by 0.02 M acetate buffer) and 32°C.

Laccase activity was assayed by spectrophotometrically monitoring the change in absorbance of the enzyme solution caused by laccase oxidation of syringaldazine substrate.[12,13] The difference in the changes in absorbance (ΔA_{530}) per minute between the enzyme and blank solution at the wavelength of 530 nm and light path of 1 cm was

monitored using a UV-2450 UV-visible spectrophotometer from Shimadzu. One unit of laccase was defined as the amount of enzyme required to produce a ΔA_{530} of 0.001/min at pH 6.5 at 30°C in a 3-ml reaction volume using syringaldazine as substrate.

Image analysis

For surface color measurement, an image scanning technique was employed. The veneer samples were scanned in three replicates before and after the experiments with Epson scanner model 2480 photo using 800 dpi resolution and an 8-bit gray scale. The digital images acquired, stored in bitmap file format (BMP), were analyzed using a computer program written specifically for this research. The program ran on MATLAB software version 7.0.4 and yielded results in number of pixels at each gray scale level. The average gray scale per pixel was calculated for each sample. The gray scale value of 0 represented the color of absolute black; the gray scale value of 255 represented the color of absolute white. The unit of gray scale of a veneer sample was defined as average gray scale per pixel. The difference between the average gray scale per pixel before and after the treatment of each experiment was recorded, and the percentage change in gray scale (a unit definition of teak veneer surface color improvement) was calculated. The higher the percent increase in gray scale, the lighter the color of the sample became.

Results and discussion

Because the combination of laccase and H_2O_2 has performed well with pulp and paper bleaching and laccase alone with the decolorization of wastewater aromatic substances, preliminary experiments were conducted to test their agents with teak veneers. Unfortunately, the use of laccase alone showed lack of capability to lighten the color of teak veneer surfaces because of repolymerization of degraded lignin (data not shown).[14] The use of H_2O_2 alone at different concentrations helped lighten the teak veneer. The higher the concentration and the longer the contact time, the lighter the teak veneer color became (Fig. 1). However, the rate of color improvement was lower as reaction time progressed. For comparison with the best result yielded by the 20% H_2O_2 concentration, the 5% and 10% H_2O_2 concentrations were selected for further experiments because the goal of this research was to reduce the amount of H_2O_2.

In many studies, the assumption that lignin was entangled with hemicellulose was reported,[2,6–8] and thus this entanglement hindered access of H_2O_2 and laccase to lignin.

To achieve our goal, hemicellulase was brought in to hydrolyze hemicellulose. Hydrolyzing hemicellulose helped expose the surface lignin to laccase and H_2O_2, providing them an easier access to lignin.[9] Therefore, experiments were performed to determine the amount of hemicellulase

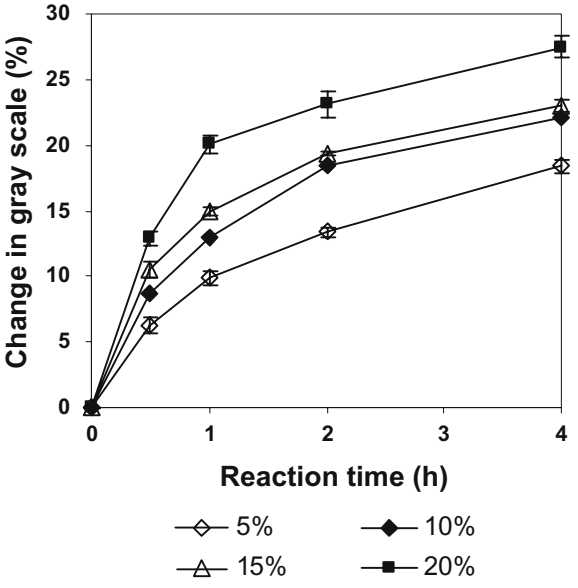

Fig. 1. Effect of hydrogen peroxide concentration and reaction time on teak veneer surface color (average % change in gray scale) at 60°C and pH 6.5

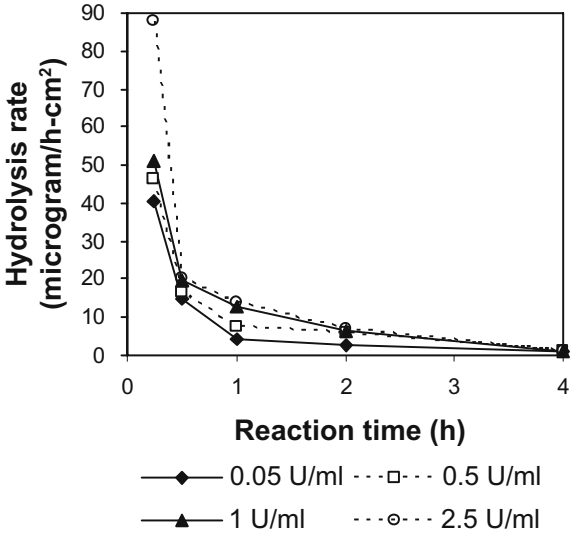

Fig. 2. Effect of concentration of hemicellulase containing xylanase and reaction time on hemicellulose hydrolysis rate of teak veneer (μg xylose equivalents/h-cm^2 veneer) at 32°C

required for removing a necessary portion of hemicellulose from teak veneer surfaces without destroying the wood texture. The rates of teak veneer hemicellulose hydrolysis, in micrograms of xylose equivalents per hour per square centimeter of veneer sample (μg/h-cm^2), are presented in Fig. 2, which shows that the rate of hydrolysis decreased remarkably as the reaction time progressed. The appropriate hemicellulase treatment condition was found to be 0.05 xylanase U/ml with 30 min reaction time at 32°C and pH 4.5, considering the conservation of material and preservation of veneer.

Because the Nelson-Somogyi method[11] gave only the total reducing sugar, high performance liquid chromatogra-

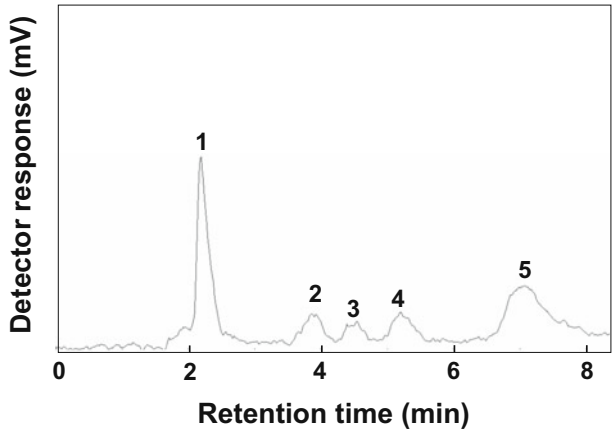

Fig. 3. HPLC chromatogram of the monosaccharides obtained from teak veneer with hemicellulase containing 0.05 xylanase U/ml for 30 min at 32°C and pH 4.5. The peaks numbered *1* to *5* refer to water, rhamnose, xylose, arabinose, and the mixture of mannose and glucose, respectively

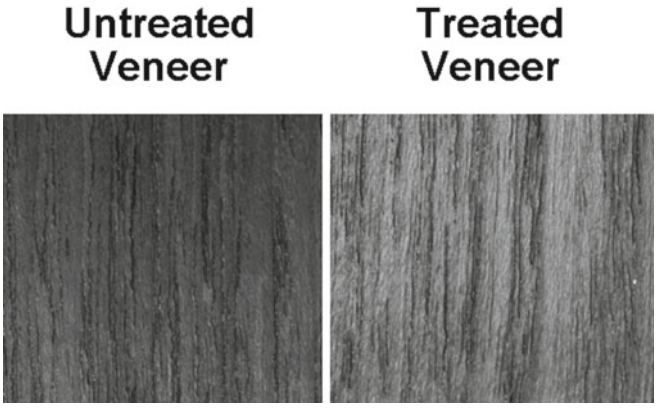

Fig. 4. Photographic comparison between untreated (*left*) and treated (*right*) veneer color with hemicellulase containing 0.05 xylanase U/ml for 30 min at 32°C and pH 4.5 followed by the mixture of 10% hydrogen peroxide and 0.05 laccase U/ml at 60°C and pH 6.5

Table 1. Effect of hemicellulase treatment followed by hydrogen peroxide or hydrogen peroxide-laccase mixture treatment on teak veneer surface color (average % change in gray scale)

Laccase(U/ml)	Change in gray scale (%)	
	5% H_2O_2	10% H_2O_2
None	19.57 ± 0.65	25.11 ± 0.44
0.01	20.10 ± 0.56	25.52 ± 0.23
0.03	21.51 ± 0.86	25.40 ± 0.46
0.05	20.61 ± 0.39	26.40 ± 0.52
0.25	19.02 ± 0.42	23.52 ± 0.70
1.00	20.40 ± 0.83	23.96 ± 0.36

Table 2. Comparison of average percent (%) change in gray scale from different experimental treatments

Method of treatment		Change in gray scale (%)
Pretreatment[a]	Treatments	
None	5% H_2O_2	18.41 ± 0.52
None	10% H_2O_2	22.13 ± 0.38
None	15% H_2O_2	23.04 ± 0.47
None	20% H_2O_2	27.48 ± 0.85
Pretreatment	5% H_2O_2	19.57 ± 0.65
Pretreatment	10% H_2O_2	25.11 ± 0.44
Pretreatment	5% H_2O_2 + 0.03 laccase U/ml	21.51 ± 0.86
Pretreatment	10% H_2O_2 + 0.05 laccase U/ml	26.40 ± 0.52
None	ECF	23.51 ± 2.62

ECF, elemental chlorine free
[a] Pretreatment was done using hemicellulase as 0.05 xylanase U/ml

phy (HPLC) was used to determine the composition of sugars released from the hydrolysis of teak veneer. The monosaccharides released, shown in Fig. 3 as the HPLC pattern of the hydrolysate, were rhamnose, xylose, arabinose, and the mixture of mannose and glucose, calculated as 3.17, 1.44, 3.70, and 3.69 μg/cm^2 veneer, respectively. Not only xylan but also glucomannan and others could be removed by the hydrolysis; this might accelerate the next treatment.

After pretreating teak veneer surfaces with hemicellulase, treatment with H_2O_2 or the H_2O_2-laccase mixture was immediately applied for veneer lightening. Table 1 shows the effect of laccase in the H_2O_2-laccase mixture. The improvement in teak veneer color was restricted at laccase concentration less than 0.05 U/ml, probably because of repolymerization of degraded lignin under the high concentration of laccase. It was also found that the best concentration of laccase was 0.03 U/ml for 5% H_2O_2 and 0.05 U/ml for 10% H_2O_2, respectively.

The representative results from different experimental treatments are presented in Table 2 for comparison. The 5% H_2O_2 treatment under the presence of 0.03 laccase U/ml after hemicellulase pretreatment yielded the teak veneer color improvement of 21.51% change in gray scale. The percent change was close to the 22.13% obtained using 10% H_2O_2 (no enzymes), showing the reduced amount of H_2O_2 requirement to be almost one-half. The pretreatment of the teak veneer with hemicellulase containing 0.05 xylanase U/ml followed by the treatment of the 10% H_2O_2 yielded the color improvement of 25.11% change in gray scale, higher than the 23.04% obtained using 15% H_2O_2 (no enzymes). It also showed a lower consumption of H_2O_2. Furthermore, when the 0.05% laccase U/ml was added into the 10% H_2O_2, the color improvement rose from 25.11% to 26.40%, close to the highest improvement, 27.48%, obtained using the 20% H_2O_2 with no enzymes and close to the target color of natural teak. This improvement was done at almost one-half of the H_2O_2 requirement.

Figure 4 illustrates a photographic comparison between the untreated and treated veneer color. The treatment was sequentially done with hemicellulase containing 0.05 xylanase U/ml at 32°C and pH 4.5 for 30 min and with the mixture of 10% H_2O_2 and 0.05 laccase U/ml at 60°C and pH 6.5 for 4 h. The color of the teak veneer was evidently lightened by the proposed method.

The elemental chlorine free (ECF) result when treated with the industrial process is included in Table 2 for com-

parison with the results of this research. This comparison was done to show that the process involving total chlorine free (TCF) would be able to compete with the process of ECF and inspire the use of a TCF method. It can be concluded that the use of a small amount of environmentally friendly enzymes improves the color of teak veneer surfaces and at the same time reduces the necessary amount of H_2O_2, supporting the objective of this research.

Conclusions

Hemicellulase treatment in sequence with H_2O_2 or a H_2O_2-laccase mixture treatment is useful for improvement of the bleachability of teak veneer surface color and at the same time for reduction in the necessary amount of the H_2O_2. Image technology can be used to measure the color improvement as percentage change in gray scale. A treatment combination of hemicellulase containing 0.05 xylanase U/ml at 32°C at pH 4.5 for 30 min in sequence with the mixture of 10% H_2O_2 and 0.05 laccase U/ml at 60°C at pH 6.5 for 4 h yields color improvement close to the target color of natural teak. The necessary amount of H_2O_2 can be reduced by almost one-half by the combined use of the environmentally friendly enzymes hemicellulase and laccase.

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