Alkaline Enzyme Treatment of Spruce Wood to Increase Permeability

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In this study, spruce sapwood was administered an alkaline enzyme treatment to improve the flow of wood liquid so that more preservative chemicals could be injected. Spruce wood is recognised as a refractory wood species. Pit membranes play an important role in liquid flow. In this study, an alkaline pectinase enzyme was applied to remove the pectin layer on the torus of the pits and margo. After enzymatic treatment, the pectin layers on the pit membrane were removed. When samples were investigated by both scanning electron microscopy (SEM) and mercury intrusion porosimetry (MIP), it was evident that pit membranes were destroyed and the permeability increased. In addition, no noteworthy weight loss was observed.

Keywords: Alkaline pectinase enzyme; Bioprep^{TM} 3000L; Mercury intrusion porosimetry; Permeability; Scanning electron microscope; Spruce wood

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INTRODUCTION

Wood is a renewable, biodegradable material that is abundantly accessible in our natural habitat. It has been an important material for mankind throughout history and has been used in countless applications, such as fuel, shelters, houses, paper-making, and the construction industry (Fengel and Wegener 1989). Because of its non-homogeneous structure, many drawbacks have been observed during experimentation. Wood is decomposed by a variety of biological agents, including fungi, bacteria, and insects (Schmidt 2006). As a result, many solutions are being sought to improve its properties, such as preservative chemicals and impregnation methods. However, there are some important problems in the wood protection area. One of the most significant problems includes refractory wood species, which have anatomical features that make the impregnation process difficult.

When the wood is intended to be in contact with the ground or kept in an outdoor area, it is impregnated with chemicals or other substances, and the objective is to have such agents penetrate deeply into the wood. Several factors, including sapwood, heartwood, density, bordered pits, tracheids, and resin canals, influence the permeability of wood (Flynn 1995). A major influence on the permeability of wood is the pit membrane, specifically, the number and size of the pit membrane pores. Spruce wood is one of the most refractory wood species; its pits tend to close below the fiber saturation point (Panek *et al.* 2013). A bordered pit structure is composed of a centralised thickened disk, called the torus, and a supporting membrane known as the margo (Comstock and Cote 1968). Studies have shown that during the process of wood drying, high surface tension causes the displacement of membranes as well as sealing off of the pit aperture. This is called pit aspiration (Bolton and Petty 1977a,b; Fujii *et al.* 1997).

When wood is dead or transformed into heartwood, pit aspiration occurs, which induces a reduction in permeability (Comstock and Cote 1968; Usta 2005). Lee *et al.* (2012) suggested that the bordered pit membrane was formed from complex material containing a fibrous structure and coated amorphous gel (pectin). Maschek *et al.* (2013) supported the suggestion that the pectin layer is dense on the surface of the torus at the unaspirated pits. They also found that after pectinase treatment, there were two distinctive cellulose layers.

To improve liquid permeability, several methods have been implemented, and these can be divided into four categories: biological, chemical, mechanical, and physical treatments, such as drying schemes, steam use, incising, and vacuum pressure treatment (Mai *et al.* 2004; Yildiz *et al.* 2012; Panek *et al.* 2013; He *et al.* 2014). One of the chemical treatment methods is enzymatic treatment. In this case, the chemical agent, pH, treatment time, and temperature also affect wood properties during the process. The wood structure can be changed by the implementation method.

Pectin is a polysaccharide substrate found in the cell walls and can be broken down by pectic enzymes (Fang 2013). Bacteria, yeast, and fungi are the production sources of the pectinase enzyme (Dosanjh and Hoondal 1996; Blanco *et al.* 1999; Hoondal *et al.* 2002). Bioprep^{TM}3000L is generated from the *Bacillus* species (Adamsen *et al.* 2002).

The alkaline pectinase enzyme takes part in many applications, such as textiles, fiber processing, industrial waste water, and coffee and tea fermentation (Hoondal *et al.* 2002). Waxy materials and pectin affect cotton fabrics' absorbency in the textile industry. To scour cotton fabrics, alkaline pectinase is the most appropriate enzyme because it does not cause cellulose degradation (Tzanov *et al.* 2001). Studies have revealed that this enzyme does not deteriorate the cellulose fiber of cotton. Alkaline pectinase is implemented under mild alkaline conditions, which are favorable for the preparation process (Etters 1999).

The objective of the present research study was to develop an enzymatic process for the improvement of the impregnation properties of spruce wood. Despite the many studies on the role of Bioprep^{TM}3000L in the textile industry, the effects of this enzyme on refractory wood species are largely unexplored. Studies have revealed that this enzyme does not degrade cotton cellulose, but it removes non-cellulosic material from the fiber, *e.g.*, fats, waxes, pectines, and proteins (Tzanov *et al.* 2001; Hashem 2007). An attempt was made to increase the permeability of spruce wood using Bioprep^{TM}3000L, which degrades pectic material on the pit membranes without damaging the wood's structure. After the enzymatic treatment, the wood structure was investigated using both a scanning electron microscope (SEM) and a mercury intrusion porosimetry (MIP) device. Enzyme effects were shown *via* SEM, and its influence on wood porosity was demonstrated through the results of MIP.

EXPERIMENTAL

Materials

Oriental spruce (*Picea orientalis* L.) wood samples selected for this study were purchased from a lumber market in Trabzon. The sapwood portions of the spruce wood were used. The lumbers were cut into pieces with dimensions of 25×15×5 mm^3 (longitudinal×radial×tangential).

Methods

Enzymatic treatment of wood

The samples were treated with an alkaline pectinase, Bioprep^{TM}3000L (Novo Nordisk, USA) Alc. Pectinase Standard Units (APSU)/g in 0.1 M phosphate buffer at pH 8 for two weeks and at 55 °C. The test samples were soaked in the sealed case with solution. The enzyme concentration was 5 g/L. The specimen/solution ratio was adjusted to 1:4(v/v), respectively. The control specimens were incubated at the same conditions. The pH of the solution was checked and adjusted everyday. After treatment, the samples were placed into boiling water for 10 min to deactivate the enzyme. The samples were then washed with cold water in a Büchner funnel. Following this, they were equilibrated in a conditioning room at 23 ± 2 ºC and 65 ± 5 relative humidity until they reached constant weight. The weight loss of the samples was calculated by their dry weight before and after treatment.

Scanning electron microscopy (SEM)

Test (treated with enzyme) and control samples were investigated using a scanning electron microscope (SEM, Zeiss Evo LS10, Germany) device. Prior to the investigation, samples were oven-dried and then coated with gold (Emitech SC7620, France). The pit degradation and microstructure of the wood were examined.

Mercury intrusion porosimetry (MIP)

MIP tests were conducted with a Quantachrome/Poremaster automatic pore size analyser (USA). Samples were cut by a scroll saw from both enzyme-treated wood and control wood with dimensions of 10×6×5mm^3 (longitudinal×radial×tangential). Control samples and four enzyme-treated samples were investigated. Before testing, they were oven dried. Measurements were performed at low and high pressure. When the pressure ranged from 0.0055 to 3.7232 MPa, the pore diameters ranged from 3 nm to 220 µm. When the pressure increased, the mercury intruded into much smaller pores. Mercury did not cause any wetting or swelling of the wood.

Pore size distribution and porosity can be calculated according to the Washburn equation (Eq. 1,Washburn 1921).

$$r = -\frac{2\gamma\cos\theta}{p} \quad (1)$$

where r is the pore radius, p is pressure, γ is the surface tension of mercury (0.48 N/m), and θ is the wetting angle of mercury (140°) (Junghans and Bächle 2005). The porosity of the samples was investigated post-test.

RESULTS AND DISCUSSION

A commercial product containing the pectinase enzyme was used in the study. Pectate lyase is a mono-component of this alkaline pectinase enzyme, and it is active in a mild alkaline medium (pH 8 to 10) that does not degrade cellulose (Agrawal *et al.* 2007). The enzyme breaks down the α-(1–4) glycosidic bonds between the galacturonic monomers that compose pectic substances (Alkorta *et al.* 1998).

The alkaline pectinase enzyme degraded the pectin layer on the torus of the pit membrane. As can be seen in Fig. 1(a), most of the pit membranes of the untreated samples

were closed. However, after enzymatic treatment, nearly all of the pit membranes were ruptured or enlarged (Fig. 1(b). Figure1(c) clearly demonstrates the deformation of the pit membrane through the use of the alkaline pectinase enzyme. After the degradation of the pit membranes, which influence the liquid transportation of wood, permeability was expected to increase. It can be expected that under pressure during the impregnation progress, ruptured pits expand or open completely.

Previous studies have revealed that the margo and torus consist of a microfibrillar structure that is covered by amorphous substances that are soluble in KOH solution (Fengel 1966; Bauch and Berndt 1973; Imamura *et al.* 1974). Maschek *et al.* (2013) revealed that when they labelled the torus of pits, the pectin layer on the torus surface was dense. They concluded that cellulose microfibrils were covered with the pectin layer. When specimens were treated with Bioprep^{TM}3000L, the pectin component of the torus was removed and the torus was ruptured (West *et al.* 2012). Likewise, Maschek *et al.* (2013) reported that the pectinase enzyme removed the pectin layer and almost all the pits were degraded.

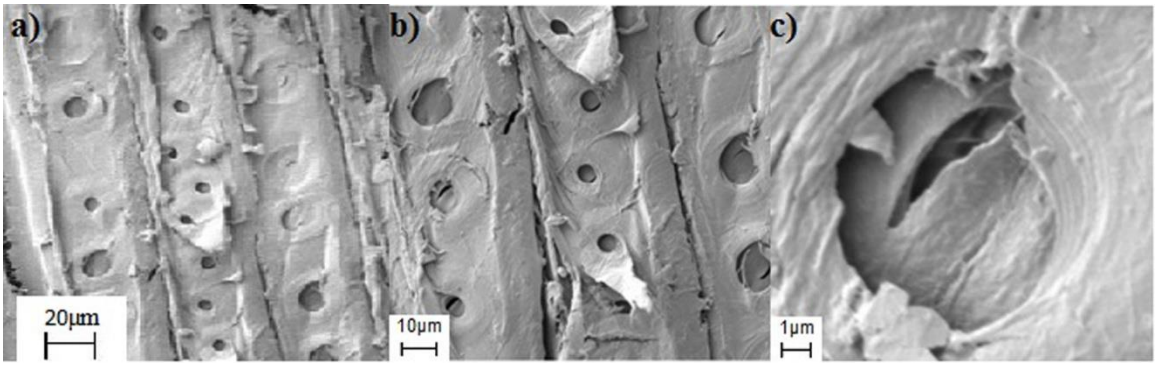

Fig. 1. SEM images of spruce wood samples: a) untreated spruce sapwood; b–c) enzyme-treated spruce sapwood.

To support the SEM results, a mercury intrusion porosimetry (MIP) test was performed. As expected, the MIP results showed that the enzyme treatment increased the permeability of spruce sapwood samples compared with untreated ones. According to Table 1, it is clear that total intrusion volume had a comparatively increased positive correlation with porosity and average pore diameter. Although total intrusion volume, pore area, and average pore diameter values also changed, the bulk densities of the control samples and sapwood samples after enzymatic treatment were almost the same.

Table 1. MIP Results for Both Enzyme-treated Samples and Untreated Samples

Samples	Total Intrusion Volume (mL/g)	Total Pore Area (m^2/g)	Average Pore Diameter (nm)	Bulk Density (g/cm^3)	Porosity (%)
Treated Sapwood	1.1260	10.3408	698.2	0.1717	19.33
Control Sapwood	0.9989	5.7666	541.8	0.1866	18.64

Pore radii were classified into three groups: r< 0.1 µm (microvoids), 0.1 to 5 µm (small tracheid gaps), 0.1 to 0.7 µm (diameter of margo capillaries), and r>5 µm (lumen radii) (Schneider 1979; Plotze and Niemz 2011; He *et al.* 2014). Figures 2 and 3 reveal a clear increase in porosity. Alterations in both cumulative pore volume and total intrusion

volume ranging from 100 to 10,000 nm occurred after enzyme treatment. However, essential differentiation took place between 100 and 1000 nm, composed primarily of pit membranes (Schneider 1979). Additionally, another important change occurred between 1000 and 50000 nm. Pores in this range are mainly lumens or checks induced by enzymatic treatment, which needs confirmation (He 2014). When the pore volume decreased to below 100 nm, effect of alkaline pectinase enzyme treatment declined, which indicates that enzyme treatment made no important contributions to the pore volume.

Figure 2 illustrates how significantly the enzyme influenced permeability. While the total intruded mercury of the enzyme-treated sample was 1.1033 at 100 nm, that of untreated samples was 0.85. Enzyme treatment had an important effect on the permeability of spruce sapwood. As enzymatic degradation was seen *via* SEM, MIP results also confirmed that sealed pits opened after alkaline enzyme treatment without damaging wood properties.

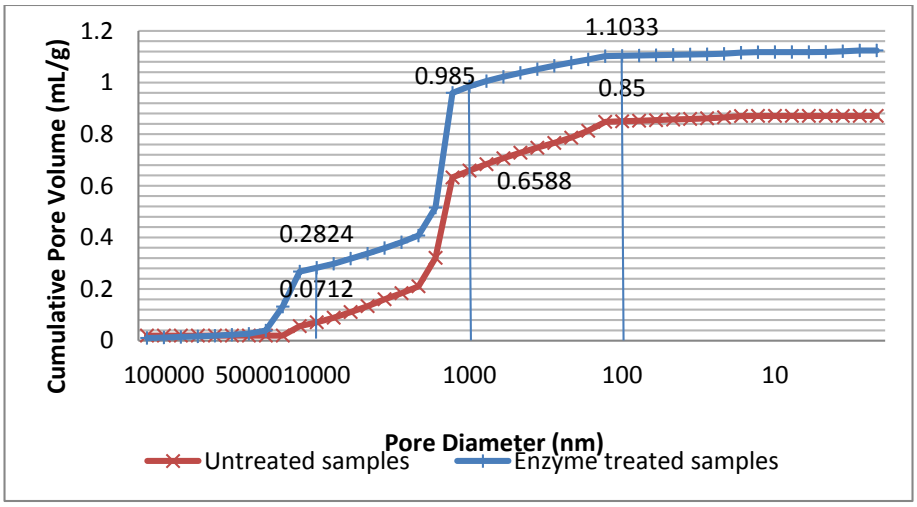

Fig. 2. Cumulative pore volume and pore diameter of spruce sapwood

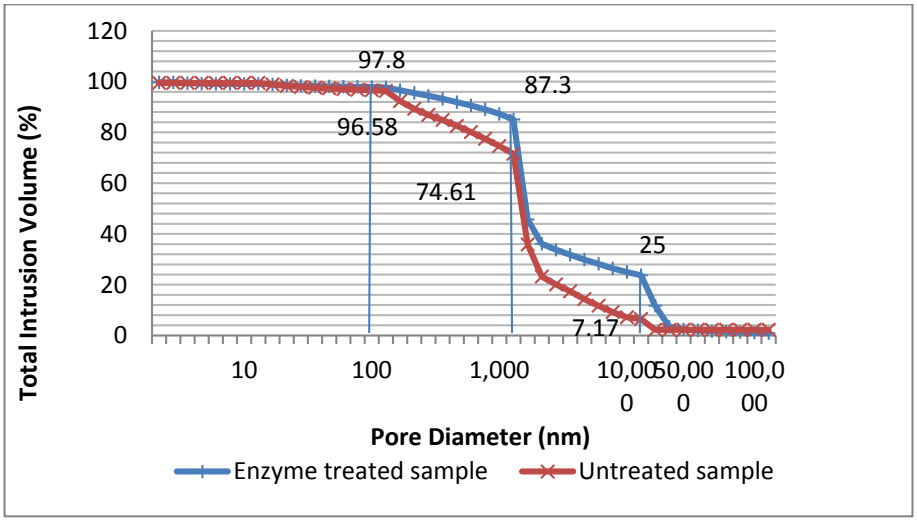

Fig. 3. Intrusion volume percentage and pore diameter of spruce sapwood

The calculated weight loss of the treated samples was less than 1%. Accordingly, it can be stated that enzyme treatment did not significantly influence the mechanical properties of wood, but further research is needed to confirm these findings.

CONCLUSIONS

1. Spruce sapwood samples were treated with Bioprep^{TM}3000L at pH 8 for two weeks and at 55 °C. Alkaline pectinase enzyme treatment changed the pit membrane structure.
2. There was no noteworthy weight loss (less than 1%) in the treated samples.
3. Both SEM and MIP results showed that pit membranes were ruptured; some of them were completely opened or enlarged.
4. The total volume of intrusion mercury also revealed the increased porosity of the wood.
5. The results of this research showed that the use of Bioprep^{TM}3000L has the potential to increase the permeability of spruce sapwood.

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