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INVESTIGATION OF ENZYMATIC EFFECTS ON PIT MEMBRANES USING LIGHT AND SCANNING ELECTRON MICROSCOPY

Spruce wood is one of the refractory wood species. Pit membranes, which provide liquid flow between the wood cells, have an influence on the permeability of wood. However, these membranes tend to close under the fibre saturation point (FSP), which makes the impregnation process more difficult. In this study, spruce sapwood samples were treated with two different commercial enzymes to improve their permeability. Bioprep 3000 L and Viscozyme L, which are mostly used in the textile industry, are alkaline pectinase enzymes and acidic pectinase enzymes respectively. Following enzymatic treatment, mass losses in the wood samples were observed and the wood samples were analysed with light microscopy (LM) and scanning electron microscopy (SEM). The mass losses of the wood samples were less than 2%. All enzyme treated and untreated wood samples were stained with toluidine blue. The pectin material was coloured red with toluidine blue stain. However, enzymatic treatment caused the loss of red coloration along with the expansion and rupturing of the pit membranes. These results showed that the pit membranes were destroyed.

Keywords: spruce wood, permeability, enzymatic treatment, light microscopy, scanning electron microscopy

Introduction

Wood is a renewable, recyclable, and biodegradable material with many advantageous features, such as its high strength properties, and its warm attractive appearance. In spite of the numerous advantages, wood is a degradable material when exposed to convenient conditions [Schmidt 2006]. Every year, billions of dollars are spent on the renovation of degraded wood. To increase its

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durability, biological, physical, and chemical modification methods are being implemented to improve its properties [Hill 2007]. Furthermore, when wood is impregnated with chemicals or other substances, it is necessary to obtain deep and homogeneous penetration.

Spruce wood is recognized as one of the refractory wood species [Liese and Bauch 1967; Flynn 1995]. The impregnation process is complicated for refractory wood species and it is difficult to achieve the necessary penetration. Sapwood, heartwood, high density, bordered pits, tracheids, and resin canals are among the factors affecting wood permeability [Flynn 1995]. The pit membrane and size of the pit membrane pores are significant to the transportation of liquid in the wood.

Water interacts with hydroxyl groups in the wood which affects the chemical reaction with wood [Rowell 2012]. As a result of this, wood drying is necessary for effective protection. However, spruce wood pits also tend to close under the fibre saturation point [Panek et al. 2013]. As water evaporates from the surface, capillary tension occurs throughout the wood drying process. In coniferous trees, the pit membranes are pressed into the pit aperture region. Hydroxyl constitutes the hydrogen bonds between the membrane and border. Torus then seals the pit aperture, which blocks the liquid flow [Thomas and Kringstad 1971; Siau 1984; Maschek et al. 2013]. The aspirated pits in the tracheid cell wall reduce the permeability of the wood, which makes the impregnation processes problematic [Siau 1984].

The aim of this study was to degrade the pit membranes using enzymatic processes in order to improve spruce sapwood permeability. Previous studies have also indicated the presence of pectin on the torus [Bauch et al. 1968; Lee et al. 2012; Maschek et al. 2013]. For this purpose, spruce sapwood samples were treated with two contrasting commercial enzymes; Bioprep 3000 L and Viscozyme L. The degradation of the pit membranes varied depending on the enzyme. After the enzymatic treatment, tools including the light microscope (LM) and scanning electron microscope (SEM) were used to investigate the enzyme effects on the wood's structure.

Materials and methods

Oriental spruce (*Picea orientalis* L.) wood grown in the Black Sea region of Turkey was used throughout this study. The sapwood blocks of spruce wood came from the outer portion of the tree with the dimensions of 25 × 15 × 5 mm^3 (longitudinal × radial × tangential), and were treated with enzymes. Before enzymatic treatment, samples were conditioned at 20°C and 65% relative humidity (RH) until they were a constant weight. Eight wood samples were selected for each enzyme. After enzymatic treatment, the mass losses of the samples were calculated from the dry weight before and after the treatment.

Two different enzymes, Bioprep 3000 L (alkaline pectinase), and Viscozyme L (acidic pectinase) were used independently in order to degrade the pit membranes. The spruce sapwood samples were treated with enzymes in a sealed case with a solution of pH 8 (adjusted using 0.1 M phosphate buffer) for Bioprep 3000 L or at pH 4.5 (adjusted using 0.1 M acetate buffer) for Viscozyme L. The enzyme concentrations were 10 g/L and the incubation time was 7 days for both enzymes. The specimen/solution ratio was adjusted to 1:4 (v/v). While the incubation temperature was set at 55°C for Bioprep 3000 L, the Viscozyme L was set at 40°C. The pH of the solutions was monitored and adjusted daily.

Wood samples were sectioned applying a sliding microtome at a thickness of approximately 60 μm and stained with toluidine blue for 1 minute and rinsed before analysis [West et. al. 2012]. A staining solution contains 0.05% of toluidine blue in 0.1 M of phosphate buffer at pH 6.8. Sections were examined with an Olympus BX50 microscope. It is known that pectic acids turn red with toluidine blue staining [O'Brien et al. 1964; Feder and O'Brien 1968].

The enzyme treated and the untreated samples were determined by a scanning electron microscope (SEM, Zeiss Evo LS10, Germany) device. For investigation purposes, the samples were first oven-dried and given a coating of gold (Emitech SC7620, France).

Results and discussion

Mass losses

The mass losses of the wood samples caused by the enzymatic treatment are shown in table 1. The enzyme complex is supposed to create more weight loss in the wood. However, the alkali pectinase enzyme induced higher mass losses compared to the acidic pectinase enzyme. Viscozyme L has a lower cellulase activity which caused less degradation in the wood [Foulk et al. 2008]. When spruce sapwood samples were treated with Bioprep 3000 L (0.5% concentration), mass losses were less than 1% [Durmaz et al. 2015]. It is also known that pectic material in fresh plant material is about 0.5-4.0% [Kashyap et al. 2000; Jayani et al. 2005]. These indicate that the mass losses of the wood samples could increase, parallel with the enzyme concentration and pectic material in the wood sample. However, enzymatic treatment might have affected

Table 1. Mass losses of wood samples

Enzymes	Mass losses (%)	SD
Bioprep 3000 L	1.75	0.13
Viscozyme L	1.69	0.10

other wood components. According to the results, bio-treatment did not cause significant mass losses in the wood samples. It was established that the mass losses caused by enzymatic treatment could not adversely affect the evaluation of the wood.

LM Imaging

The toluidine blue staining of both the enzyme treated and untreated samples was to determine the pectin degradation. As can be seen in figure 1, the differences between the enzyme treated and untreated samples is remarkable. One can see that the pit region and torus were stained with toluidine blue in the untreated sample (fig. 1a). The red colouring of the pit membrane shows the presence of pectic materials. Furthermore, previous studies have also revealed that the pectic material, which also covers the torus surface, is located in the pit membrane structure [Bauch et al. 1968; Imamura and Harada 1973; Tschernitz and Sachs 1975; Fengel and Weneger 1989; Jayani et al. 2005; Lee et al. 2012; Maschek et al. 2013].

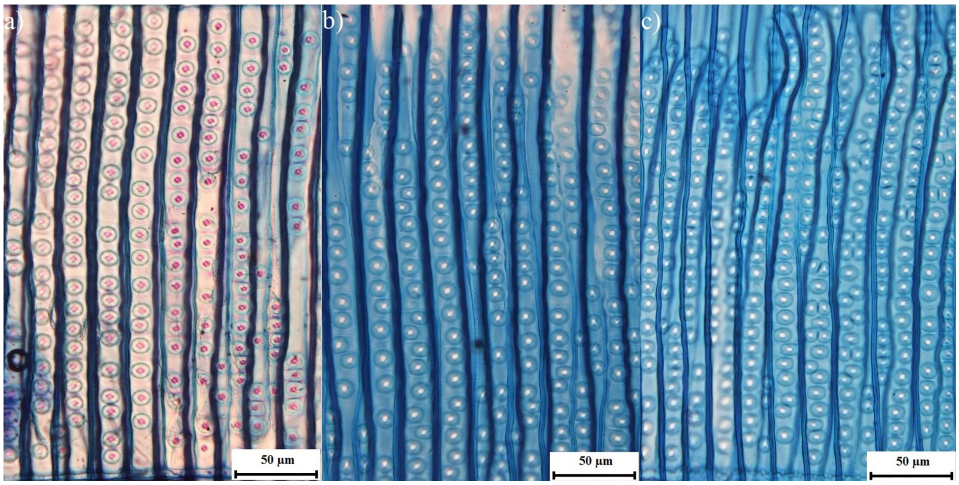

Fig. 1. LM images of spruce wood samples: a – radial section of control sample, b – radial section of samples treated with Bioprep 3000 L, c – radial section of samples treated with Viscozyme L

When the images of the enzyme treated samples are examined, the changes to the pit membrane structures are clearly visible (fig. 1b-c). The toluidine blue did not stain the pit membranes of the enzyme treated samples red. Additionally, the absence of red staining in the pit region and torus indicates the degradation of the pectic materials. Previous studies have also confirmed that the wood samples were treated with pectinase enzymes, however, the pectin layer was removed and the pit structures were degraded [West et al. 2012; Durmaz et al. 2015].

SEM Imaging

The enzyme treated and untreated samples were investigated using scanning electron microscopy to evaluate the degradation of the pit membranes (fig. 2). The SEM images of the treated samples indicate clearly the enzyme effects on the pit membranes. As seen in figure 2a, more than half of the pits were closed in the untreated samples. However, the enzymatic treatment changes this as the pit structures were degraded following enzymatic treatment. An image of a sample treated with Bioprep 3000 L shows the enzyme effect in which the pit membranes were ruptured (fig. 2b). Previous studies have shown that the Bioprep 3000 L enzyme degraded the pit membranes also causing a rupture on the torus [Imamura et al. 1974; West et al. 2012; Durmaz et al. 2015]. As can be seen in figure 2c, Viscozyme L degraded the pit membranes more than that of the Bioprep 3000 L enzyme. Almost all of the pits were deformed through the use of the acidic pectinase enzyme. Unlike Bioprep 3000 L, torus was completely degraded, causing the pit membranes to open following acidic pectinase enzyme treatment.

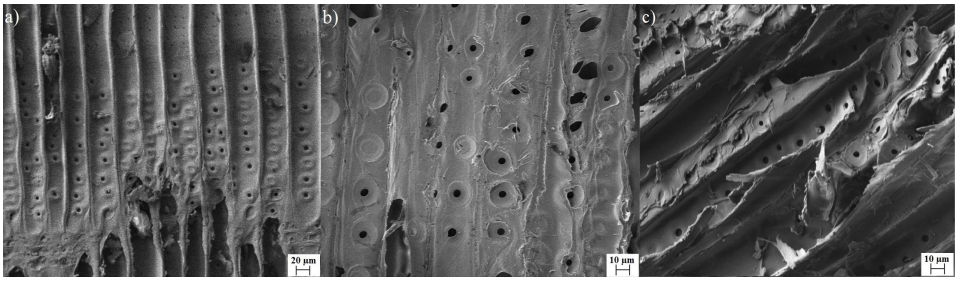

Fig. 2. SEM images of spruce wood samples: a – radial section of control sample, b – radial section of samples treated with Bioprep 3000 L, c – radial section of samples treated with Viscozyme L

Bioprep 3000 L is composed of pectinase enzyme alone, which breaks down the pectic substance to monomers [Alkorta et al. 1998]. Due to the formation of single enzymes, the effect of the Bioprep 3000 L enzyme was superficial. In contrast, Viscozyme L is a multi-enzyme complex which is composed of cellulase, hemicellulase, arabinase, and pectinase [Foulk et al. 2008]. Therefore, the severity of the deformation increased significantly. In spite of the enzymatic treatment, individual closed pits were observed. Following aspiration, incrustation of the pit membranes occurs as a result of extractives accumulation [Fengel 1970; Siau 1984]. The enzyme effects changed, based on the intensity of incrustation.

Conclusions

The objective of the present study is to show the effects of enzymes, which are widely used in the textile industry, on the pit structures of spruce sapwood. For this purpose, enzyme treated and untreated samples were examined using light and scanning electron microscopy. Enzymatic treatment did not cause very great mass losses in the wood samples. The weight losses were less than 2%, which indicate that the main components of the wood could not be significantly degraded. Untreated wood samples, in which more than half of the pits were observed to be closed, were first stained with toluidine blue. As in previous studies, pectic material was removed via enzymatic treatment from the pit structure and this was confirmed by the absence of stained pit membranes. Furthermore, the SEM images indicated deformation on the torus, and degradation to the pit membrane caused by both of the enzymes. The effect of enzymes depends on their components. According to the results of this study, the named enzymes have a high potential to increase the permeability of spruce sapwood.

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