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Oxygen-scavenging coatings and films based on lignosulfonates and laccase

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ABSTRACT

Laccase and lignosulfonates were included in coating colors and embedded in latex-based or starch-based films and coatings on foil or board. After 6 days at 23 °C and 100% relative humidity, the oxygen content in airtight chambers decreased from 1.0% (synthetic gas consisting of 99% N_2 and 1% O_2) to 0.3% in the presence of board coated with lignosulfonate and laccase, while the oxygen content remained unchanged in control experiments without enzyme. The water stability of lignosulfonate-containing latex-based coatings and starch-based films was improved after laccase-catalyzed oxidation of lignosulfonates, which indicates polymerization to products with lower solubility in water. Furthermore, the E' modulus of starch-based films increased with 30%, which indicates laccase-catalyzed polymerization of lignosulfonates resulting in increased stiffness of the film. The results suggest that laccases and lignosulfonates can be used as an oxygen-scavenging system in active packaging and that enzyme-catalyzed polymerization of lignosulfonates contributes to improved water stability and mechanical properties.
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1. Introduction

The market for food packaging exhibits constant growth. Research in the field of food packaging needs to address sustainability, for example by aiming at reducing the amount of non-renewable raw material used (Pynnönen and Heiskanen, 2008). Although utilization of renewable bio-polymers in dispersion coating of food-packaging materials is advantageous with respect to sustainability, main disadvantages compared to petroleum-based alternatives include poor mechanical properties and poor water stability. To overcome these disadvantages, attempts have been made to modify bio-polymers chemically and to cross-link the bio-polymer chains (Kim and Lee, 2002; Yoon et al., 2007; Reddy and Yang, 2010). Barrier properties and mechanical strength have been found to be improved by blending the starch with mineral filler, such as clay (Schuman et al., 2005; Wilhelm et al., 2003).

The shelf-life of food can be improved by using active packaging. Oxygen scavengers are often used in active packages to protect the food against deteriorative oxidation processes, such as lipid and vitamin oxidation. There are several kinds of oxygen scavengers, among which iron-based substances are used most frequently. Oxygen-reducing enzymes, primarily glucose oxidase, have been shown to have potential to catalyze oxygen scavenging in food packages, as they can be directly incorporated into a coating color applied onto the board surface (Nestorsson et al., 2008; Johansson et al., 2011). Laccase has also shown potential for use as oxygen scavenger in active packaging (Chatterjee et al., 2011).

Laccase (EC 1.10.3.2) is a copper-containing enzyme that catalyzes a one-electron oxidation of phenolic hydroxyl groups to phenoxy radicals, while oxygen is reduced to water (Widsten and Kandelbauer, 2008; Rodríguez Couto and Toca Herrera, 2006; Nyanhongo et al., 2011).

$$4\mathrm{PhOH} + \mathrm{O_2} \xrightarrow{\text{Laccase}} 4\mathrm{PhO^{\bullet}} + 2\mathrm{H_2O}$$

The phenoxy radicals subsequently form quinones or polymerization products. In a previous study based on small phenolic compounds, three different laccases were immobilized through entrapment in a dispersion coating applied on paper board (Chatterjee et al., 2011). Laccase from the white-rot fungus *Trametes versicolor* was found to have interesting properties, such as the capability to efficiently oxidize phenolic compounds that were less suitable as substrates for the other laccases examined. The present investigation focuses on the use of *T. versicolor* laccase as an oxygen scavenger together with a polymeric substrate, namely lignosulfonates. Immobilization was performed by entrapment of the enzyme and the substrate in dispersion coatings based on latex or starch.

Lignosulfonates are products from wood biorefineries based on sulfite pulping processes. The molecular weight of lignosulfonates varies widely, typically in the range 5000–60,000. They consist of a hydrophobic backbone of phenylpropane units with hydrophilic sulfonate and hydroxyl groups. They are known to act as a

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plasticizer for starch and are likely to form molecular connections (e.g. hydrogen bonds) with starch chains (Baumberger, 2002). Laccase oxidizes lignosulfonates to radicals, which then polymerize through radical coupling (Areskogh et al., 2010; Nyanhongo et al., 2011). Since polymerization may affect the properties of films and coatings, we have investigated the water stability and the mechanical properties of the laccase–lignosulfonate system. Starch has the advantage that it is a renewable bio-polymer, but it would be desirable to improve the water stability and the mechanical properties of starch-based films.

In this investigation, we have also explored the activity of boards coated with laccase and lignosulfonates during incubation under varied relative humidity (RH). The water activity (a_w) of a food is the ratio between the partial water vapor pressure of the food, and the vapor pressure of distilled water at the same temperature and external pressure (Chirife and Fontana, 2008). The water that is available in a system, i.e. the a_w of the system, may be important for solubilizing and mobilizing enzymatic substrates, and for obtaining a correct enzyme molecular conformation. Enzymatic activity generally increases with increasing a_w (Acker, 1969; Lee and Kim, 1995; Bell, 2008), and most enzymatic reactions are rapidly slowed down below an a_w of 0.8 (Chirife and Fontana, 2008). Previous studies of enzyme-containing films have been based on experiments in which the films have been immersed in aqueous solutions for determination of activity (Nestorsson et al., 2008; Johansson et al., 2011; Chatterjee et al., 2011), but in this investigation we have measured oxygen consumption in airtight chambers, which is an advantageous approach considering that it permits investigation of the effect of RH on the enzymatic activity.

2. Materials and methods

2.1. Materials

Enzyme: Laccase from *Trametes* (syn. *Coriolus*, *Polyporus*) *versicolor* was obtained from Jülich Fine Chemicals GmbH (Jülich, Germany). The crude laccase preparation supplied by the manufacturer was used without further purification. *Polymers and chemicals*: Lignosulfonate was kindly provided by Domsjö Fabriker (Örnsköldsvik, Sweden). The styrene-butadiene latex (SB-latex) was supplied by Styron Europe GmbH (Horgen, Switzerland). According to the manufacturer, the SB-latex has a glass transition temperature (T_g) of 6 °C. The dry-solids content (SCAN-P 39:80) and the pH were measured prior to use and found to be 50% and 5.5, respectively. The clay (Barrisurf LX) was supplied by Imerys Minerals Ltd. (Cornwall, UK). The starch, Perlcoat55 (hydroxypropylated and oxidized potato starch), was supplied by Lyckeby Industrial AB (Kristianstad, Sweden). Glycerol with a purity of 99.5% was supplied by Karlshamns Tefac AB (Karlshamn, Sweden). Magnesium chloride was obtained from Riedel-de Haën (Seelze, Germany), magnesium nitrate was obtained from Merck (Darmstadt, Germany), potassium chloride was obtained from Fluka (Buchs, Switzerland), and 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer was obtained from Sigma–Aldrich (St. Louis, MO, USA). *Carriers for coatings*: Two different kinds of carriers for the coatings were used: (I) Skultuna Aluminum Folie FRYS, an aluminum foil coated on one side with polypropylene (PP), which was obtained from Skultuna Folie AB (Skultuna, Sweden), and (II) Enso Prime Barr, a three-ply packaging barrier board, which was supplied by Stora Enso Imatra (Imatra, Finland). The top side of the board is coated with polyethylene (PE) and the reverse side is coated with PE/ethylene vinyl alcohol (EVOH). The board was used in experiments with oxygen scavenging in airtight containers, while the carrier based on aluminum foil, which does not swell in water, was used in experiments with water stability of latex-based coatings.

Table 1
Coating-color recipes.

Component	Latex-based coating[a]	Starch-based coating[b]
Latex	100	–
Starch	10	100
Clay	55	55
Lignosulfonate	30	30
Glycerol	–	30
Enzyme preparation	6	6

[a] Parts of the component per hundred parts (by weight) of dry latex.
[b] Parts of the component per hundred parts (by weight) of dry starch.

2.2. Enzyme activity assays

Units of activity: One unit (U) of laccase was defined as the amount of enzyme needed to catalyze the oxidation of 1 μmol of pyrogallol per min in a reaction performed at pH 6.5 and at a temperature of 25 °C. Although the optimal pH for oxidation of phenolic substrates is around 4 (Hong et al., 2006), *T. versicolor* laccase is most stable at pH 6–7 (Larsson et al., 2001), and pH 6.5 was used also in the subsequent experiments. *Reaction with lignosulfonates in buffered aqueous solution*: To evaluate the reaction conditions and how laccase affected the lignosulfonates, reactions were performed with 100 mg/ml lignosulfonates, 2.5 U/ml laccase, and 100 mM MOPS buffer (pH 6.5). 7 ml of the reaction mixture were transferred to two glass vials that were subsequently placed in a heating block at 37 °C under stirring. After 24 h, the reaction mixture was freeze-dried using a Heto Drywinner (Heto-Holten S/S, Allerød, Denmark). Lignosulfonates that had not been treated with enzyme were used as control. The freeze-dried samples were analyzed (in duplicates) through size-exclusion chromatography by MoRe Research AB (Örnsköldsvik, Sweden).

2.3. Enzyme immobilization

Preparation of coated board and foil: A starch solution with 20% dry-solids content was prepared by keeping a mixture of starch and water in a boiling water bath for 45 min under vigorous stirring followed by rapid cooling to room temperature. The clay was dispersed in deionized water with a conductivity of 1 μS/cm to a final solids content of 63% according to the manufacturer's protocol. The coating color was prepared by mixing latex with clay, freshly cooked starch, and lignosulfonates in the proportions described in Table 1. The pH was adjusted to 6.5 (using a 1 M solution of NaOH) and the coating color was subsequently left to stir for 15 min prior to addition of laccase in a concentration of 0.91 U/g wet coating color. As a control, coating color without enzyme was prepared. In order to obtain sufficient adhesion between the PE-laminated board and the coating color, the board was corona-treated by using a laboratory-scale corona equipment (Corona-Plus, Vetaphone, Kolding, Denmark) prior to coating. The corona power output was 60 W min/m^2. Ceramic electrodes and an aluminum roll with a perimeter speed of 50 m/min were used. The corona-treated PE-side of the board and the PP-side of the PP/aluminum foil were double coated using the bench coater K202 Control Coater (RK Print-Coat Instruments Ltd., Royston, UK), which was fitted with a wire-wound bar giving a nominal wet deposit of 24 μm. The drying conditions were 105 °C for 30 s followed by 30 °C for 24 h to ensure complete film formation of the latex. *Casting of films*: To evaluate the effect of the enzymatic reaction on the water stability of biodegradable starch films, free films without latex were produced by casting in Petri dishes. The coating color was prepared by mixing the freshly cooked starch with clay and glycerol in the proportions indicated in Table 1. The pH was adjusted to 6.5 (using the 1 M solution of NaOH) prior to addition of the lignosulfonates. The coating color was subsequently left to stir for 15 min prior

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