

Pitch reduction with the white-rot fungus *Ceriporiopsis subvermispora*

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SUMMARY

This research was conducted to examine the ability of the white-rot fungus *Ceriporiopsis subvermispora* to reduce the resin components (mainly the triglycerides) of loblolly pine wood chips. Wood chips were inoculated with mycelium of *C. subvermispora* and incubated for 2 weeks. After extraction with dichloromethane the resins were analyzed with gel permeation chromatography and gas chromatography. It could be shown that the triglycerides, which are mainly responsible for the formation of troublesome pitch, as well as other resin components are degraded.

INTRODUCTION

Pitch causes a number of technical problems in pulp and paper manufacture (1). The composition of the pitch (the hydrophobic extractives of wood such as triglycerides, sterols, sterol esters, resin acids, fatty acids, etc.) changes during the year and varies from wood species to wood species (2). Pitch-related problems are always associated with a relatively high content of triglycerides in the pitch (3).

Currently there are two commercial biotechnological approaches to ameliorating the pitch problem. The first approach is to use lipase to hydrolyze the triglycerides after chemical or mechanical pulping (4 - 6). This process takes only about 2 to 3 hours and leads to a 86 % reduction of the triglycerides and about a 30 % reduction of the adhesiveness of the pitch (4); adhesiveness is related to the most troublesome problems caused by pitch. The second biotechnological approach is to inoculate wood chips with a naturally occurring, non-pathogenic wood-colonizing fungus, *Ophiostoma piliferum*, which consumes the pitch. This process takes about 14 days, and, in pine chips, decreases both the total resin and the triglyceride content of the chips (7 - 10). *O. piliferum* is marketed as Cartapip[®] by Sandoz Chemicals Corporation, Charlotte, NC.

Our recent studies (11) have shown that the pitch content of wood chips is also reduced during an experimental "biopulping process" with the white-rot fungus *C. subvermispora*. Biopulping is the treatment of wood chips with a lignin-degrading fungus such as *C. subvermispora* prior

to pulping. Such pretreatment reduces mechanical pulping energy consumption and improves certain paper strength properties (12 - 14). The aim of the present study was to investigate the changes in wood resin composition, mainly the triglycerides, during the growth of the fungus *C. subvermispora* in wood chips.

MATERIALS AND METHODS

Fungus. *Ceriporiopsis subvermispora* CZ-3 (14,15) was obtained from the Center for Forest Mycology Research, Forest Products Laboratory, Madison, WI, U.S.A.

Wood chips. Loblolly pine (*Pinus taeda* L.) was obtained from the Talladega National Forest in Talladega, AL, U.S.A. Fresh logs were debarked and chipped to a nominal size of 16 mm. These chips were frozen immediately after chipping to prevent the growth of microorganisms.

Inoculum preparation. The culture medium (1.2 l) contained 28.8 g.l⁻¹ potato dextrose broth (Difco Laboratories, Detroit, MI) and 8.72 g.l⁻¹ yeast extract (Amberex 1003, Universal Foods Corporation, Milwaukee, WI). Four Fernbach flasks (2.8 l) each containing 300 ml of medium were autoclaved for 20 min at 121 °C, cooled, and inoculated with 30 plugs cut with a 9 mm diameter cork bore from 10-day-old petri plate cultures on potato dextrose agar. The flasks were incubated at 27 °C and 65 % relative humidity for 10 days without agitation. The spent medium from four cultures was decanted, the mycelial mats washed with sterile water, and aseptically blended in a Waring blender. Sterile water was added to obtain 400 ml of mycelial suspension, 100 ml of which was used to inoculate each bioreactor containing 1500 g of chips (dry weight basis). This amount of inoculum was 1.5 g of fungus (dry weight basis) per bioreactor.

Chip preparation and bioreactor inoculation. Thawed chips were mixed thoroughly and placed in static bed bioreactors (12). The bioreactors were then autoclave for 90 min at 121 °C. The above inoculum was then used to inoculate these bioreactors, bioreactors containing non-inoculated chips served as controls. The final water content of the chips was adjusted to 55 % (wet weight basis) with sterile water. The bioreactors were then sealed, shaken vigorously, and incubated at 27 ± 1 °C for 14 days. Each bioreactor received a continuous supply of humidified air at a rate of 0.02 l/min (12).

Harvest and extraction. Three samples (100 g wet weight each) were taken from each bioreactor after incubation. These samples were freeze-dried and ground to pass a 2 mm screen in a Wiley mill. Samples were extracted with dichloromethane for 6 h following the TAPPI standard T204om-88. Solvent was removed in a rotary evaporator at 70 °C, and the resins were dissolved in 5 ml tetrahydrofurane. A 125 µl sample was taken for HPLC analyses. The solvent was then removed from the remaining sample at 60 °C.

HPLC. The tetrahydrofurane was removed in a stream of nitrogen, and the remaining resins dissolved in 2 ml tetrahydrofurane (16); 2.5 mg of vanillin in 0.5 ml tetrahydrofuran was added as internal standard. Gel permeation chromatography employed a Hewlett Packard 1050 pump, a Waters R401 refractive index detector and a Hewlett Packard HP 3396 II integrator. The separations were performed on two Ultrastyrigel columns (Millipore Corporation, Waters Chromatography Division, Milford, MA). 300 by 7.8 mm, with a pore size of 50 and 10 nm, connected in series with a Shodex (Millipore Corporation, Waters Chromatography Division, Milford, MA) GPC KF-800P precolumn. Runs were also made with the standards triolein,

oleic acid, cholesterol, cholesteryl palmitate and abietic acid (1 mg.ml⁻¹ each). Dilutions of these standards ranging from 0.1 to 2.5 mg.ml⁻¹ were used to calibrate the peaks for quantitation.

Gaschromatography. Sterol and fatty acids appeared together as a single peak in the HPLC chromatogram. It was therefore necessary to analyze the fatty acids separately (17). 100 mg of chip resin was dissolved in 1 ml of diethylether and methanol 9 : 1 (v/v). Diazomethane was added until no further nitrogen was produced. The reaction mixture was kept under nitrogen for 1 min without agitation, and solvents were removed in a 70 °C water bath under a nitrogen stream. Samples were then dissolved in 5 ml methyl-tert-butylether. This solution was used directly for gas chromatography. The separation was performed on a Hewlett Packard HP 5890 gas chromatography fitted with a HP 7673A auto sampler, a flame ionization detector, and an HP 3396 integrator. Injector and detector temperatures were 280 °C, the injection volume was 1 µl, the split rate at the injection port was 50:1. Temperature programming for the 30 m x 0.25 mm DB-225 (J & W Scientific, Folsom, MA) column with 0.15 µm film thickness was from 160 °C to 220 °C at 10 °C.min⁻¹. A solution of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachic acid (C20:0), behenic acid (C22:0), lignoceric acid (C24:0) and hexacosanoic acid (C26:0), all in concentrations of about 1 mg.ml⁻¹, was methylated as above and used to identify and calibrate the peaks. Total fatty acids were estimated by adding the values for each fatty acid, determined from peak areas based on standards.

RESULTS AND DISCUSSION

Reduction of total resin content. The total resin content (DCM-extractables) of the wood chips was reduced from 2.64 ± 0.15 % to 1.93 ± 0.21 %, which is a relative reduction of 27 % within two weeks.

Reduction of resin components. The main interest of this study was to determine the changes in the triglyceride content of the resins. Figure 1 shows the HPLC chromatogram of native resin from loblolly pine. It can be seen in table 1 that *C. subvermispora* degraded 53 % of the triglycerides within two weeks.

	<i>Control</i>	<i>C. subvermispora</i>
Triglycerides	0.30	0.14
Sterol esters	0.13	0.04
Fatty acids	0.33	0.14
Sterols	0.19	0.01
Resin acids	0.77	0.46

Table 1: Changes in resin components during a 2-week treatment of loblolly pine chips with *C. subvermispora*. All numbers are calculated on dry wood basis.

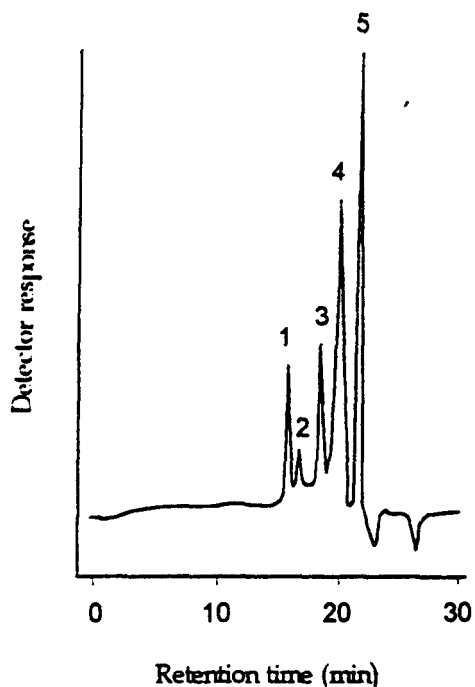


Figure 1: Gel permeation chromatogram of native extractives from loblolly pine (1: triglycerides, 2: sterol esters, 3: sterols and fatty acids, 4: resin acids, 5: vanillin).

Moreover, a significant reduction in all the other resin components which could be detected by HPLC or GC was observed (Table 1). Fatty acids and sterols were eluted in the same peak at the HPLC analysis. Therefore, the fatty acids were analyzed separately by gas chromatography. Figure 2 shows a gas chromatogram of the methyl esters of the fatty acids in native loblolly pine resin.

This study shows that the experimental biopulping fungus *Ceriporiopsis subvermispota* is also effective in reducing the amount of pitch in loblolly pine chips. This adds another advantage to the already known effects of a pretreatment of loblolly chips with *C. subvermispota*, such as energy savings and improvement of pulp quality (11).

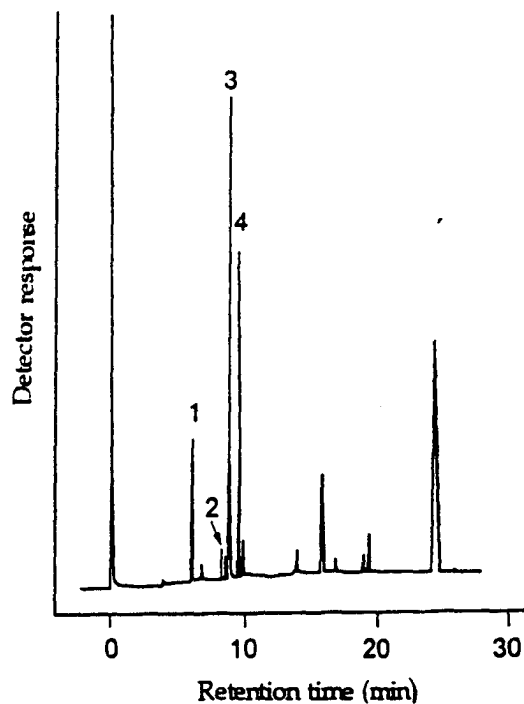


Figure 2: Gas chromatogram of methyl esters of the fatty acids in native loblolly pine resin. (1: palmitic acid, 2: stearic acid, 3: oleic acid, 4: linoleic acid).

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