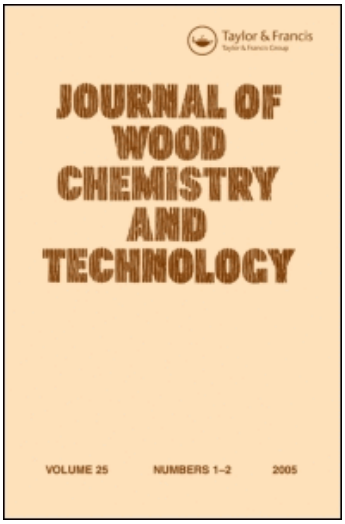

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FRACTIONAL SEPARATION OF MIDDLE LAMELLA AND SECONDARY WALL TISSUE FROM SPRUCE WOOD

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ABSTRACT

Differential sedimentation of finely ground wood has been used to isolate fractions of black spruce in which the lignin content varied from 20 to 60 percent. The fractions of low lignin content consisted mainly of secondary wall tissue while those of high lignin content came predominantly from the compound middle lamella.

INTRODUCTION

It is becoming increasingly apparent that the chemistry of wood polymers is not uniform but varies in different morphological regions of wood. Most of the evidence for this has been microscopic[1-6]. Few attempts have been made to isolate wood components from well defined morphological regions. In 1936, Bailey[7] isolated 0.5 mg of middle lamella lignin by a method so laborious that no other researcher has attempted to repeat the work. In 1961, Meier[8] studied the distribution of polysaccharides in hardwood and softwood fibres by removing layers of cells near the cambium at different stages of maturation. By a chemical peeling technique, Luce[9] determined the radial variation in content of hemicelluloses in softwood fibres.

By differential extraction of lignin and chemical characterization of the fractions, Kolar et al.[10] have studied the distribution of syringyl and guaiacyl residues in birch lignin. Lindström

and Glad-Normark[11] noted that the fines from unbleached kraft pulps from Scotch pine contain up to 50% lignin and that these fines contain fragments of cell corner which are responsible for the high lignin content. However, this cell corner lignin is probably altered during the kraft cook.

Recently Hardell et al.[12] have developped a method of separating wood particles into fractions of fibres, ray cells and fines, which contained secondary wall, ray cell and compound middle lamella lignins respectively. They applied the method to both spruce[12] and birch wood[13] and detected certain chemical differences in the lignins from the different morphological regions which confirm earlier results obtained by ultraviolet microscopic methods[5,6]. The method developed by Hardell et al.[12] involved fractionation of thermomechanical pulp and disintegrated wood by a combination of sieving and gravitational sedimentation. Ray cell particles were separated manually by rotation in a liquid on a watch glass.

The purpose of the work described in the present paper was to develop a simple and reproducible method of preparing centigram-scale fractions of wood tissue from the compound middle lamella and secondary wall of black spruce. The method is based on the difference in densities between lignin and carbohydrate material. The wood is ground to a fine powder, suspended in a separation medium and the suspension is centrifuged. The lignin is of lower density ($\rho \approx 1.4$ g.mL^{-1}) than the carbohydrates ($\rho \approx 1.5$ g.mL^{-1})[14]. If the proper density of the separation medium is chosen, only particles from the compound middle lamella, which will be high in lignin content, will float and the other particles will sink. Conversely, the density of the medium may be set so that only the particles from the secondary wall, which will be lowest in lignin content, will sink while all others will float.

In their previous work, Hardell et al.[12,13] noted some ambiguity in their results for lignin concentration. In the present work, the concentration of lignin was determined by four different methods. In addition, the fractions obtained were examined microscopically and characterized by ultraviolet and infrared spectroscopy.

EXPERIMENTAL

Preparation of Wood Flour

Freshly cut logs of black spruce [Picea mariana (Mill.)B.S.P.] were debarked by hand. The green wood was chipped in two stages. In the first, a No. 24 Appleton Chipper was used, which gave wood chips approximately 5 cm square by 0.5 cm thick. These chips were then re-chipped in a Mead Chipper, Size 2, giving chips about 1 cm long, 0.3 cm wide, and 0.1 cm thick. The chips were then air-dried for five days and ground to a fine, flour-like material in a Bauer No. 730 Hurricane Pulverizer. The Hurrican Pulverizer has a refiner-type grinding action and incorporates a recycling system for particles that are too large.

The resulting wood-flour was about the same colour as the original spruce wood and contained many small fibre fragments and fines but no large pieces of wood.

The wood flour was then extracted with acetone for 16 h. The lignin content of the dry extractive-free wood flour was found by the Klason technique to be 26.8% (grams lignin per gram extractive-free wood).

Approximately 30 g of dry wood flour was then placed in a 21-cm diameter sieve containing a 500-mesh screen. The hole size of the screen was 25 μm. The sieve was covered and placed on the Eberbach horizontal shaker for 15 min at full speed. The powder that passed the screen, approximately 30% of the original wood flour, was collected and saved as the working material. It had a Klason lignin content about 2% higher than the original wood flour.

Fractionation

The 500-mesh wood flour was a pale cream colour and contained no large fibrous pieces. Approximately 2 g of the wood flour was placed in a 250 mL jar and 100 mL of separation liquid added. The separation liquid was a mixture of carbon tetrachloride (tetrachloromethane) and 1,4-dioxane, adjusted to give the desired density. The density was measured gravimetrically in a calibrated volumetric flask at constant temperature.

The resulting mixture was treated in a MSE Ultrasonic Power Unit for five minutes. The mouth of the vessel was sealed with Parafilm to prevent the escape of vapours from the vessel as the solutions warmed upon ultrasonication. The vessel was removed, covered and cooled to room temperature. The wood flour was well dispersed.

The mixture was then transferred by pipette to glass centrifuge tubes (Figure 1) specially designed to allow the collection of a small amount of floating material without stirring up material at the bottom of the tube. The tubes were placed in a swing-bucket rotor (SW-25.1) and spun in a Beckman Model L Ultracentrifuge for 15 min at 10,000 rpm at room temperature and under vacuum. It was noted that the particles flocculated after the mixture stood for periods of over 1 h. This was avoided by gentle stirring of the mixture between ultrasonication and centrifugation.

After centrifugation, the tubes were carefully removed and the floating material collected by pipette. The sample was placed in a sample vial and the solvent evaporated in a vacuum oven at 60°C. In the isolation of samples of secondary wall lignin the same procedure was followed except that the wood flour was not sieved and the fraction which sank during centrifugation was collected.

The most important factor in obtaining reproducible samples was found to be the maintenance of constant density in the separation medium. The density of the carbon tetrachloride:dioxane mixtures varied considerably with temperature; it was essential that the experiments be carried out in a room with a temperature variation of no more than plus or minus 2°C, with less variation preferable.

Measurement of Lignin Content

The measurement of the lignin content of the isolated samples was carried out by four methods: a micro-Klason technique, infrared absorption, solid state ultraviolet absorption, and ultraviolet absorption of samples dissolved in a mixture of acetyl bromide (ethanoyl bromide) and acetic acid (ethanoic acid). For each method,

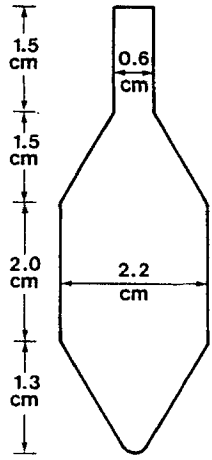

FIGURE 1. Glass centrifuge tubes used to collect fractions of wood material. These specially designed tubes were made to order by a glass blower.

at least ten measurements were made on the wood flour and the reproducibility calculated as a standard deviation.

The micro-Klason technique was the same as the usual Klason analysis[15] except that samples as small as 1 mg were analyzed. From one to ten milligrams of dry extractive-free wood tissue were weighed accurately in a 10 mL, round-bottomed flask. For each milligram of wood, 0.015 mL of 72% sulfuric acid was added to the wood using a microburet. The wood was thoroughly mixed with the acid using a glass stirring rod and the mixture allowed to stand at 20°C for 2 h. Enough water was added to make a 3% sulfuric acid solution and the mixture then refluxed for 4 h. It was important that the condenser used for the refluxing be the same scale as the round-bottomed flask, as a significant amount of water in the form of droplets was found to stick to the inside of a large condenser raising the acid concentration above 3%. After refluxing, the mixture was cooled and filtered, with care being taken to ensure that all the material was transferred to the filter. Selas silver filters (0.8 μm) were used for the fil-

tration. It was important that the water and sulfuric acid be filtered at least 3 times to ensure that small insoluble particles did not interfere with the results. After the lignin was filtered, it was washed with 1 L of distilled water and then dried in a vacuum oven at 60°C for 24 h. The lignin was then weighed. As a control in the micro-Klason technique, a sample of black spruce with a known Klason lignin content was analyzed. The results agreed with the normal Klason analysis to within ±2%.

For the infrared analysis the absorbance at 1510 cm^{-1} due to the aromatic stretching of carbon-carbon bonds was used. One to two milligrams of sample were pressed in a pellet with KCl and the peak height at 1510 cm^{-1} measured in the same manner as the method of Kolboe and Ellefsen[16]. Samples of black spruce wood of varying mass were used to calibrate the technique. The method was found to be reproducible to within ±1.5%.

For ultraviolet measurements in the solid state the method of Bolker and Somerville[17] was adopted. The finely divided sample was pressed in a KCl pellet, similar to the type used for infrared spectroscopy. Pellets containing from 0.1 to 2.0 mg of tissue were prepared. The absorbance at 280 nm was measured for each sample and Beers law was found to be obeyed in this range. Black spruce of known Klason lignin content was used as a control. This technique provided a fast, convenient method for measuring lignin contents on very small samples with a reproducibility of ±2%.

The lignin contents of the tissue fractions were also measured by the method of Johnson *et al.*[18] in which the wood sample is dissolved in a mixture of acetyl bromide and acetic acid. One to three milligrams of sample were placed in a reaction vessel with 2 mL of 25% acetyl bromide in acetic acid. The vessel was placed in an oil bath at 70°C. The method calls for a reaction time of 30 min but it was found that for samples high in middle lamella lignin not all the lignin dissolved after this period. For this reason a reaction time of 2 h was used. With black spruce wood flour of known Klason lignin content as a control it was possible to measure the lignin content

of the various tissue fractions with a reproducibility of ±1%. The reaction time of 2 h produced an absorptivity of 27.3 $cm^{-1}g^{-1}L$ for the lignin. This was higher than the value of 23.5 $cm^{-1}g^{-1}L$ found after 0.5 h reaction time, in agreement with the observations of Johnson et al.[18].

As discussed in the next section of the paper, the agreement between the four methods was good. The three spectroscopic methods were much more convenient to use than the micro-Klason technique. Therefore, on the tissue samples of the secondary wall, only the spectroscopic methods were used.

RESULTS AND DISCUSSION

The fractionation of black spruce wood was carried out at various densities and the lignin contents of the fractions obtained are given in Table 1. As shown in Table 1, there is good agreement between the lignin contents measured by the different methods, the maximum deviation being ±3%.

In Figure 2, the average values of lignin content and yield are plotted against the density of the separation medium. There was a gradual increase in lignin content of the floating fraction as the density was decreased from 1.460 to 1.420 $g.mL^{-1}$. This was as expected. However, it is interesting to note that the lignin content decreases below a separation density of 1.420 $g.mL^{-1}$. If lignin was the only low density component, the lignin content of the samples should increase until a density was reached at which nothing floated. The maximum in Figure 2 suggests that there is another component (or components) of wood which is of density low enough to interfere with the isolation of the compound middle lamella lignin. There seems to be only a very small amount of this material and so it interferes significantly only at low densities where the quantity of tissue in the fraction is small. The nature and origin of this light, non-lignin substance is unknown, but it could have been derived from some relatively minor wood component such as cutin[19]. It should be noted that in Table 1 Klason lignin contents are not shown for samples isolated at densities lower

TABLE 1

Lignin Analyses and Yields for Various Fractions of Black Spruce

Density of Separation ($g.mL^{-1}$)	Klason Lignin (%)	Infrared Lignin (%)	UV Pellet Lignin (%)	Acetyl Bromide Lignin (%)	Average Lignin (%)	Yield[b] (%)
1.459 (floats)	31.8	31.2	29.7	30.1	30.7 ± 1.0[a]	61.5
1.448 (floats)	37.1	36.8	34.4	33.2	35.4 ± 1.9	22.0
1.439 (floats)	41.3	43.2	37.0	40.5	40.5 ± 2.6	8.3
1.435 (floats)	44.3	42.1	43.6	42.1	43.0 ± 1.1	4.6
1.430 (floats)	40.8	42.2	39.2	37.9	40.0 ± 1.9	1.9
1.425 (floats)	45.6	45.0	38.9	42.6	43.0 ± 3.0	0.5
1.423 (floats)	50.3	48.3	49.5	48.3	49.1 ± 1.0	0.5
1.420 (floats)	52.4	52.1	50.7	51.1	51.6 ± 0.8	0.4
1.415 (floats)	-	44.6	46.9	42.3	44.6 ± 2.3	0.3
1.410 (floats)	-	36.4	35.7	31.4	34.5 ± 2.7	0.2
1.459 (sinks)	-	20.8	20.8	21.7	21.1 ± 0.5	2.3
1.455 (sinks)	-	22.4	22.3	22.8	22.5 ± 0.3	26.9
1.450 (sinks)	-	26.0	24.9	25.0	25.3 ± 0.6	52.1
1.440 (sinks)	-	27.7	25.7	25.3	26.2 ± 1.3	78.0

a) Standard deviation between the different methods.

b) Yield from sieved wood flour for floating fractions and from non-sieved wood flour for sinking fractions.

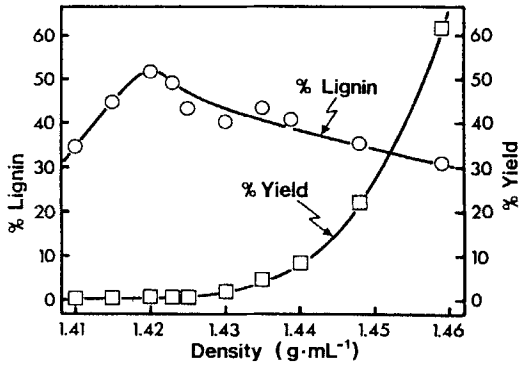

FIGURE 2. Plot to show average lignin concentration and yield of floating tissue fractions collected at various densities of the separation medium.

than 1.420 $g.mL^{-1}$. This is because the non-lignin material interfered with the Klason test and gave a result which was too high in comparison with the other three methods.

The presence of the light non-lignin substance made necessary an extra step in the isolation procedure for the compound middle lamella tissue. A sample with a lignin content of 52% which had been separated at a density of 1.420 $g.mL^{-1}$ was re-separated at densities from 1.410 to 1.418 $g.mL^{-1}$. Fractions containing 55 to 65% lignin (measured by the infrared and acetyl bromide methods) were obtained in the sediment. The floating fraction had a lignin content of about 35 to 45%. This result indicated that the light non-lignin material was not bound to the middle lamella fragments and could be separated by flotation.

It is interesting to note that Fergus et al.[3] found the lignin content of the compound middle lamella to be 50% and that of the true middle lamella to be 85%. The tissue fraction with a lignin content of 65% must have contained a considerable proportion of the true middle lamella.

Optical microscopy confirmed that the particles of the high lignin content fraction indeed came from the compound middle lamella.

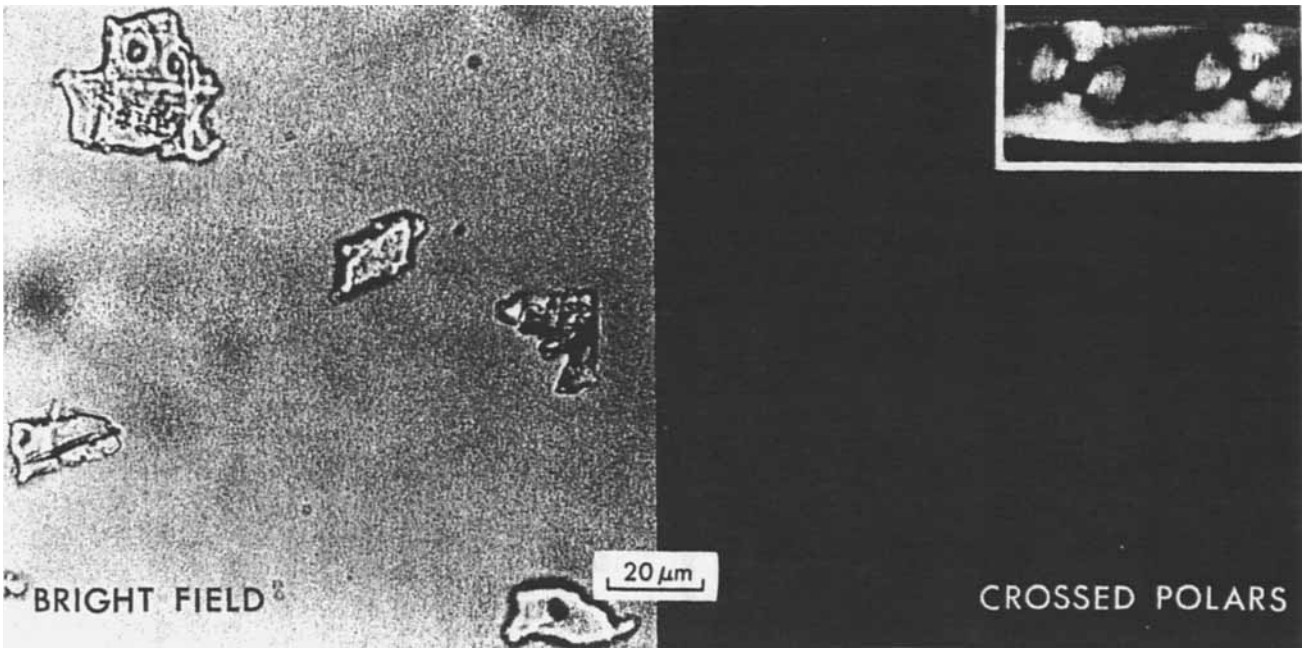

FIGURE 3. Particles of compound middle lamella in bright-field and under crossed polars. The insert in the upper right-hand corner shows bordered pits in a fibre fragment through crossed polars. Note the maltese cross image.

Figure 3 shows the middle lamella tissue under bright-field and through crossed polars. It can be seen from the bright-field photomicrograph that the particles are thin and flat, which is the shape of the compound middle lamella in wood. Through crossed polars, the image of the particles is virtually invisible, indicating that most of the material is amorphous. It should be noted that some of the middle lamella particles have small holes in them, due to the bordered-pit structure of the fibres. Bordered pits in wood fibres seen through crossed polars (Figure 3, insert) give the characteristic maltese cross image due to the circumferential alignment of the cellulose microfibrils around the pits. Such maltese crosses were completely absent when holes in the compound middle lamella tissue were viewed through crossed polars.

In Figure 4 the lignin content and the yield of the sinking fractions are plotted against the density of the separation medium. It can be seen that at a density of 1.461 $g.mL^{-1}$ a sample containing only 21% lignin was isolated. Fergus et al.[3] found the lignin concentration in the secondary wall to be 22%; this fraction must therefore be composed entirely of pieces of secondary wall.

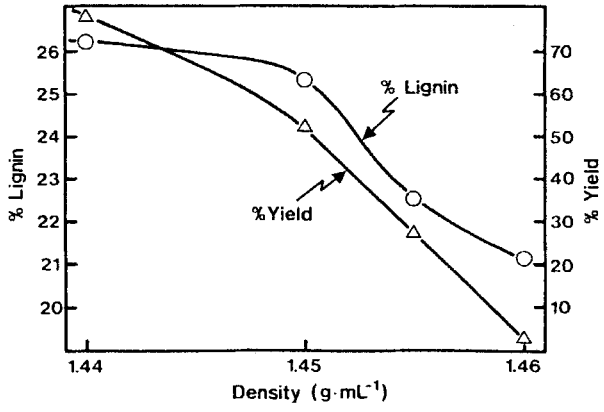

FIGURE 4. Plot of average lignin content and yield of sinking tissue fractions at various densities of the separation medium.

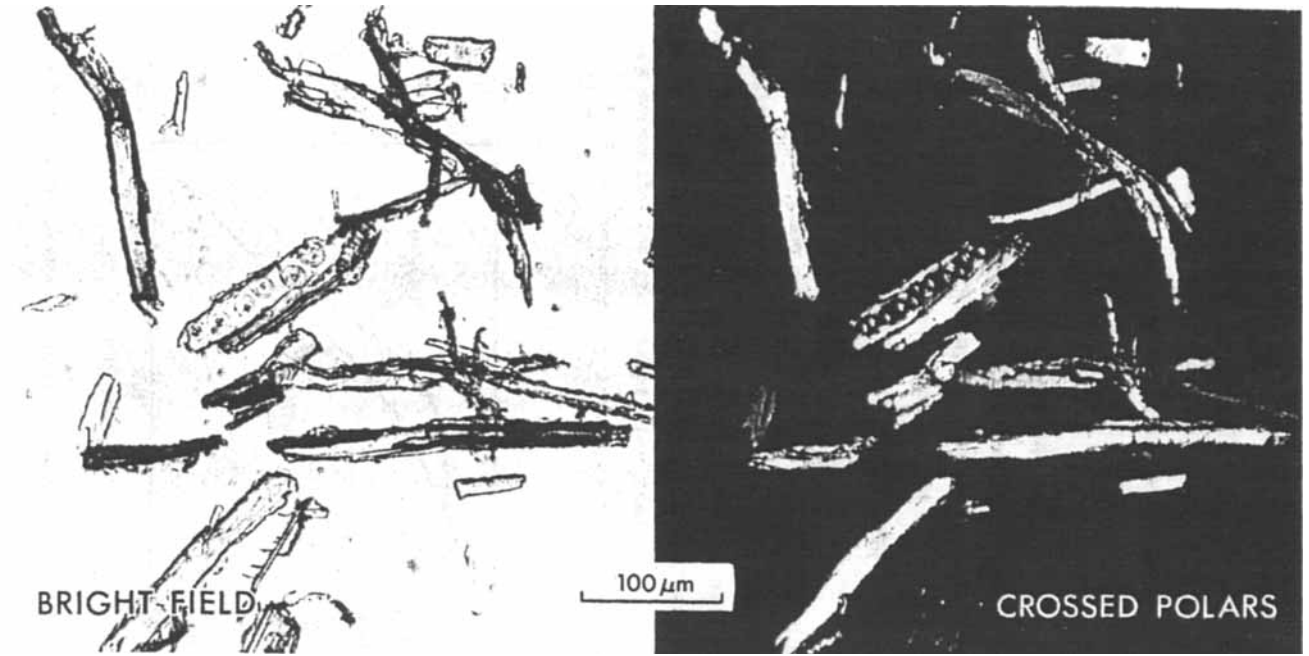

FIGURE 5. Secondary wall particles in bright-field and through crossed polars. Note the presence of maltese crosses around the bordered pits.

As viewed with the microscope, the tissue fractions that sank were found to contain few or no fragments of compound middle lamella. Figure 5 shows a sample containing 21% lignin. In contrast to the material shown in Figure 3, it resembles the fragments of a fibre wall. Under crossed polars, most of the particles give a bright image, indicating the presence of cellulose.

The floating tissue fractions, which contained a high percentage of lignin, were further characterized by infrared and ultraviolet spectroscopy. The infrared spectra showed a band at 1510 cm^{-1} (the aromatic carbon-carbon stretching frequency) which is characteristic of all lignins. The basic overall spectrum was very similar to that of other lignin preparations, especially the difference spectrum of lignin in wood[16]. The ultraviolet spectrum of the compound middle lamella sample showed absorption bands at 250 and 280 nm, characteristic of lignin.

CONCLUDING REMARKS

It has been shown that the separation of spruce wood into fractions of high and low lignin content is possible by taking advantage of the difference in density between lignin and carbohydrate material. It has also been shown that the fractions high in lignin content are rich in compound middle lamella tissue, and those low in lignin content are rich in tissue from the secondary wall. These preparations should prove to be useful to wood chemists studying the differences between middle lamella and secondary wall lignin.

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