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Determination of Lignin in Non-Wood Plant Fiber Sources

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Abstract: Lignin contents of representative non-wood plants were determined by a 2-hr treatment at 5°C with 80% sulfuric acid. Representative genera were selected to give data on stem fibers, leaf or hard fibers, and forages. The results were compared with those of a 16-hr, 72% sulfuric acid procedure from the literature. Relative standard deviations for the materials by the two methods are comparable; i.e., ±2.41–3.48% and ±2.74–3.60% with 80 and 72% acid, respectively. Analysis of variance showed significant differences between the materials at the 99% confidence level. However, at the 95% confidence level, differences due to method were found only for hard-fibered leaf material. The considerable time savings afforded by the 2-hr (5°C), 80% sulfuric acid method is desirable. The 80% sulfuric acid method has been adopted in our laboratory as the one of choice for determining lignin in fibrous non-wood plants.

Keywords: Manila hemp · Bagasse · Bamboo · Cornstalks · Crotalaria* · Fescue* · Fibers · Henequen* · Kenaf · Lignins · Non-wood plants* · Plants (organisms) · Ramie · Sisal · Sorghum* · Straw · Sudan grass* · Vegetable fibers

A STUDY by Whitehead and Quicke (*1*) emphasizes the diverse values for lignin content obtained for given materials by different analytical procedures. Pearl (*2*) and Whitehead and Quicke (*1*) also stress the importance of defining lignin as determined by a prescribed method. Procedures for determining lignin in annual non-woody agricultural products are largely modifications of those developed for analysis of woods and woody products. Since February 1962, we have been using an 80% sulfuric acid procedure (2 hr at 5°C) to determine lignin in annual plants and fibrous agricultural residues (*3*, *4*). This affords considerable time saving over the more common 16-hr, 72% sulfuric acid treatment of Sherrard and Harris (*5*) or the similar 18-hr, 72% sulfuric acid method of Peterson *et al.* (*6*). These longer (16–18 hr) methods should be contrasted with the 2-hr, 72% sulfuric acid treatment at 18–20°C developed by the Forest Products Laboratory for use on wood. This latter procedure was adopted as a standard method (T 13 m-54) by TAPPI. Although woods were used to develop this TAPPI procedure, we have applied it to annual vegetative materials, but our values were not consistent for these materials. The purpose of this paper is to report the exact procedure used in our 2-hr (5°C), 80% sulfuric acid method and the studies that led to its adoption.

RESULTS AND DISCUSSION

To establish our procedure, selected conditions for isolation and recovery of lignin from vegetative materials were evaluated. With wheat straw as standard material, we investigated such variables as digestion temperature (5–60°C), sulfuric acid concentration (50–80%), ratio of acid volume to sample weight (10–50 ml/g), digestion time (2–16 hr), coagulation acid concentration (2–6%), and coagulation time (0.25–4 hr). Determination of lignin by direct isolation and recovery of the residue from strong sulfuric acid was rejected because some lignin apparently was soluble under these conditions. This solubility was indicated by the following observations: Residue yields were lower than expected. When the strong acid filtrate was diluted to 3% acid concentration and vigorously boiled, a precipitate formed. This solid gave the characteristic rose color for lignin when treated with chlorine followed by sodium sulfite (*7*). The methoxyl content of this precipitate varied from 10 to 16%. The weight of this solid added to that of the material recovered from the strong acid approaches the value of lignin as determined by the 16-hr (10°C), 72% sulfuric acid method. These facts suggest strongly that this precipitate from dilute acid was lignin.

Similar to results of Armitage *et al.* (*8*), we found only small differences in the lignin values determined under varied acid concentrations during coagulation. The effect of coagulation time (0.25–4 hr) with 3% sulfuric acid had little influence on the lignin determination. With 80% sulfuric acid the acid-to-sample ratio 20 ml/g gave results equivalent to a 40 ml/g ratio with 72% sulfuric acid.

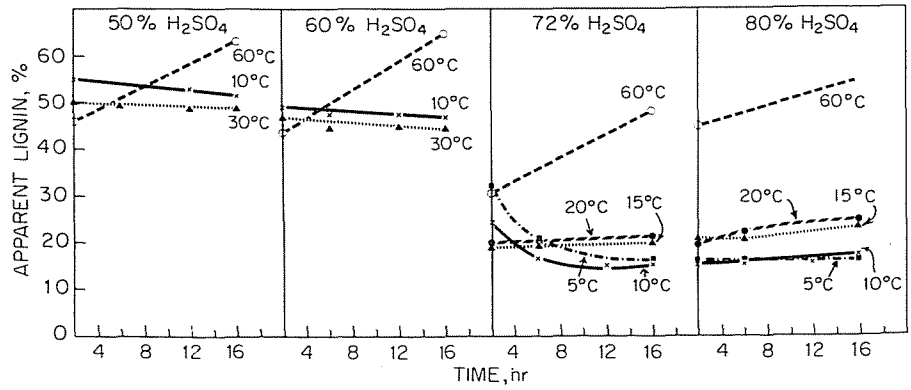

Fig. 1. Effect of time, digestion temperature, and acid concentration on apparent wheat straw lignin.

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Table I. Lignin Determination with 72% (3) and 80% Sulfuric Acid Methods

Material	Source (variety)	Lignin, % 72%, 16 hr at 10°C	Lignin, % 80%, 2 hr at 5°C
Stem-fiber			
Bagasse	Florida	19.6	19.4
Bagasse	Hawaii	21.3	21.8
Bagasse	Mexico	18.3	18.7
Bagasse	Louisiana	20.2	20.3
Bagasse	Florida	18.8	19.7
Bagasse	Philippines	20.1	19.8
Bamboo[a]	Mississippi (*Arundinaria gigantea*)	22.2	19.5
Cornstalks	Iowa	14.7	14.7
Cornstalks	Israel (hybrid)	15.3	13.7
Cornstalks	Israel (yellow dent)	13.0	16.3
Broom cornstalks	Illinois	17.3	18.5
Crotalaria	South Carolina	22.4	23.0
Kenaf fiber	Florida	10.9	10.1
Ramie fiber	Florida	20.6	21.4
Barley straw	Nebraska (Exond)	15.4	17.1
Oat straw	Illinois (Clinton)	17.7	17.4
Rice straw	Louisiana (Zenith)	12.7	14.2
Rye straw	Minnesota (commercial)	18.0	19.1
Wheat straw	Illinois (Kawvale)	15.9	15.7
Wheat straw	Nebraska (Pawnee)	18.2	18.5
Wheat straw	Illinois (Pawnee)	20.0	20.1
Wheat straw	North Dakota (Premier)	16.3	17.4
Wheat straw	Washington (Rex)	16.4	17.8
Wheat straw	North Dakota (Stewart Durum)	16.2	16.5
Wheat straw	Kansas (Tenmarq)	17.0	16.3
Leaf or hard-fiber			
Abaca	Philippines (commercial)	9.6	10.1
Henequen	Yucatan (commercial)	8.8	9.7
Sisal	Africa (commercial)	7.1	7.8
Forage fiber[b]			
Fescue	Kentucky (K34)	5.6	5.4
Fescue	Kentucky (K34)	5.4	5.6
Sudan grass	Illinois (Piper)	6.8	6.7
Sudan grass	Illinois (Piper)	6.8	6.9
Sudan grass	Illinois (Piper)	7.0	6.7
Sorghum	Illinois (Atlas)	7.9	7.9

[a] This material ground to pass a U. S. standard 40 screen.
[b] Acid-pepsin pretreatment used with all forage materials.

Table II. Precision of the 72 and 80% Sulfuric Acid Lignin Methods

	Wheat straw		Abaca		Fescue	
	72% Method	80% Method	72% Method	80% Method	72% Method	80% Method
Number of determinations	23	16	14	12	8	8
Mean	16.2	16.1	9.5	10.3	5.0	5.4
Standard deviation	0.58	0.56	0.26	0.33	0.18	0.13
Relative standard deviation (% of mean)	±3.58	±3.48	±2.74	±3.20	±3.60	±2.41

The effects we found of acid concentration, duration, and temperature on digestion prior to coagulation are shown in Fig. 1. The coagulation step for these studies involved boiling for 3 hr with 3% acid. Condensation products (*9*) probably account for the consistently higher values and the increase with time observed at 60°C.

Enzymatic prehydrolysis for removing interfering substances is essential when analyzing forages or other green materials high in protein. The pepsin-0.1*N* hydrochloric acid digestion of Ellis (*7*) was used with the forage-type materials in this study. This pepsin pretreatment has been routinely used in subsequent analyses of green non-woody fibrous plants (*3*, *4*).

Three major types of raw materials—stem fiber, leaf fiber, and cordage fiber—were used in an extensive comparison of our 2-hr (5°C), 80% sulfuric acid method and the 16-hr (10°C), 72% sulfuric acid procedure of Sherrard and Harris (*5*). Fiber sources and selected data are summarized in Table I. Results of statistical treatment of these comparative data are presented in Table II. Relative standard deviation for the three types of materials with the two methods are comparable. Time saved by the 2-hr (5°C), 80% sulfuric acid method makes it a desirable improvement over the 16-hr, 72% sulfuric acid procedure. Analysis of variance of data in Table I reveals no significant difference between the two methods for analyses of stem fiber or forage fiber. For the small sampling of leaf or hard-fiber, there is a significant difference (95% confidence level) between the methods. However, the absolute differences are small and could be fortuitous. As expected, a highly significant difference (99% confidence level) is observed between materials.

Historically (*10*), methoxyl content was one of the first methods for quantitation of lignin. The measure of methoxyl is frequently used to establish quality of lignin isolable by given procedures and, to some extent, to establish quantitative values (*1*, *10*, *11*). Although suggestive of lignin content obtained from materials listed in Table I, methoxyl values ranging from 8 to 21% in this study were more variable than are the gravimetric determinations of lignin.

EXPERIMENTAL

Materials

Most raw materials were collected at maturity. However, sugarcane bagasse was the commercial by-product produced from the milling of sugarcane harvested for optimum sugar yield. Ramie and kenaf bast fibers were obtained as textile fibers from a decorticating operation. The forages were harvested green and subsequently field-dried. Sufficient quantities of representative materials were prepared in a Wiley-type mill with 1-mm holes.

Two-Hour (5°C), 80% Sulfuric Acid Procedure

An appropriate quantity of milled air-dry solids is extracted for 8 hr in a Soxhlet with alcohol-benzene (1 : 2 v/v). The resulting lipid-free residue is allowed to air-dry overnight. Duplicate 1.000-g samples are weighed into 100-ml beakers. The contents of each beaker are cooled to 5°C (±0.1°C) and then 20 ml of cold (5°C) 80.0% (w/w) sulfuric acid is added. Each sample is triturated with a glass rod to assure maximum mixing. When required because of protein content as described by Ellis (*7*), the dry lipid-free residue is digested with pepsin before treatment with 80% acid. After digesting for 2 hr, each product is quantitatively flushed from the beaker and rod into a 2-liter Erlenmeyer flask (29/42 ⅋) with sufficient water to give a mixture con-

taining 3% sulfuric acid. The flasks are fitted with a 3-ft air condenser, and then the contents are refluxed for 1 hr to coagulate the lignin. The precipitates are allowed to settle and the hot solutions are decanted through tared Bitumen Gooch crucibles containing pads of long-fibered asbestos or ash-free papers. The precipitates are transferred to the filters, washed with hot water (>90°C) until free of acid, and oven-dried (105°C) to constant weights. Corrections for ash contents are determined from residues after ignition at 800°C. Lignin values are derived from differences in weights before and after ignition.

Ancillary Procedures

The 16-hr (10°C), 72% sulfuric acid procedure used for comparison was conducted as described by Sherrard and Harris (*5*).

Methoxy was determined on the lignin by the method of Clark (*12*).

Pretreatment with pepsin-0.1*N* hydrochloric acid (*7*) was used with green material.

LITERATURE CITED

1. Whitehead, D. L. and Quicke, G. V., *J. Sci. Food Agr.* **15**: 417 (1964).
2. Pearl, I. A., "The Chemistry of Lignin," New York, Marcel Dekker, Inc., 1967, pp. 37–58.
3. Clark, T. F. and Wolff, I. A., *Tappi* **52**: 2111 (1969).
4. Clark, T. F., Cunningham, R. L., Lindenfelser, L. A., Wolff, I. A., and Cummins, D. G., *Tappi*, "Non-wood Fiber Pulping, A Progress Report," CA Report No. 34, New Orleans, La., Nov. 2, 1970, pp. 107–132.
5. Sherrard, E. C. and Harris, E. E., *Ind. Eng. Chem.* **24**: 103 (1932).
6. Peterson, C. J., Walde, A. W., and Hixson, R. M., *Ind. Eng. Chem. Anal. Ed.* **4**: 216 (1932).
7. Ellis, G. H., *J. Assoc. Agr. Chem.* **32**: 287 (1949).
8. Armitage, E. R., Ashworth, R. de B., and Ferguson, W. S., *J. Soc. Chem. Ind.* **67**: 241 (1948).
9. Brauns, F. E., "The Chemistry of Lignin," New York, Academic Press, Inc., 1952, p. 134.
10. Brauns, F. E., "The Chemistry of Lignin," New York, Academic Press, Inc., 1952, pp. 10 and 169.
11. Moon, F. E. and Abou-Raya, A. K., *J. Sci. Food Agr.* **3**: 595 (1952).
12. Clark, E. P., *J. Assoc. Offic. Agr. Chem.* **15**: 136 (1932).

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