A COMPARATIVE STUDY OF THE WILLIAMS-OLMSTED AND DAVIS-MILLER METHODS FOR THE DETERMINATION OF LIGNIN IN PLANT TISSUES

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The wide adoption of the newer methods for the determination of indigestible residue in feeds and feces has been hindered by the lack of accurate methods for the determination of lignin. There are, seemingly, no functional groups in the lignin molecule which are directly related to the amount of lignin present. Therefore, no quantitative estimation can be based on a procedure involving this principle. The result is that most of the methods for the determination of lignin depend upon characteristic solubilities. The method most generally used involves the use of 72 percent sulfuric acid by weight with subsequent weak acid hydrolysis. The insoluble portion is termed lignin.

It is generally accepted that for the determination of lignin the material to be analyzed should be relatively free from protein, since otherwise the protein-lignin complex is weighed as apparent lignin. Pretreatments, both biological and chemical, have been recommended to accomplish this purpose. Williams and Olmsted (1935) used digestion with pancreatin as a preliminary treatment. Horwitt, Cowgill and Mendel (1936) used digestion with pepsin, diastase, and trypsin. Crampton and Maynard (1938) used peptic digestion. Norman (1936) extracted and hydrolyzed the sample before the lignin determination. Davis and Miller (1939) used pepsin, clarase, and trypsin followed by hydrolysis and extraction with 5 percent H_2SO_4 before the determination of lignin.

It has been indicated by Norman (1937) and Norman and Jenkins (1934) that the presence of pentoses in the strong H_2SO_4 digest also increases the apparent lignin yield. In this strong acid solution pentoses slowly form furfural, which condenses with the lignin molecule causing a precipitate which is weighed as lignin. The extent of this reaction is greatly reduced by holding the strong acid digest below 10° C. This cold acid hydrolysis has been employed by Williams and Olmsted (1935) and Hilpert and Littman (1935). Norman and Jenkins (1934) showed that the presence of pentoses in 72 percent H_2SO_4 for long periods of time caused high apparent lignin values even though the temperature was controlled. He also showed that after pretreatment with 3 to 5 percent acid it is of little moment whether the duration of contact with 72 percent acid be 2 or 16 hours. This method was used by Davis and Miller (1939) as a pretreatment before subjecting the sample of indigestible residue to the 72 percent acid hydrolysis. In this paper the method of Williams and Olmsted (1935) for the determination of lignin is compared with that of Davis and Miller with special emphasis on the presence of pentoses as a possible explanation for the difference in apparent lignin yields.

PROCEDURES

Williams and Olmsted Method. A pancreatin-sodium chloride solu-

tion is added to the finely suspended ether-extracted sample contained in a 50 ml flask. The system is buffered to a pH of 8 with bile buffer consisting mostly of sodium taurocholate and potassium acid phosphate. The materials are well mixed and incubated for 3 days at 45° with occasional shaking. The residue is filtered through silk cloth, washed and weighed as indigestible residue. To this is added 20 ml of chilled 68 or 72 percent H_2SO_4 (by weight). The material is shaken briskly and placed in an ice box for 24 hours, after which it is added to sufficient water to give a 4 percent acid concentration It is refluxed for 3 hours and filtered hot through alundum crucibles, weighed, ignited and reweighed. Loss on ignition is reported as lignin.

Davis and Miller Method. This method differs from the Williams and Olmsted method as follows:

1. In place of the pancreatin-sodium chloride digestion a pepsin, clarase and trypsin digestion is used.

2. The residue from the enzymatic treatment is subjected to 5 percent H_2SO_4 hydrolysis before lignin is determined.

3. The indigestible residue is treated in an ice bath with 72 percent H_2SO_4 for 15 minutes, and then for 45 minutes at room temperature in preference to the long low-temperature treatment of Williams and Olmsted.

EXPERIMENTAL

It was the aim of this research to determine the difference in apparent lignin yields given by the two methods in question and to determine whether these yields are affected by the presence of hemicellulose or pentose sugars during the strong acid hydrolysis. For this reason it was desirable to apply acid treatments according to these two methods to an indigestible residue prepared by the same enzymatic digestion; then differences in apparent lignin values would be caused by variations in acid treatment. It has been shown by Davis and Miller that the enzymatic treatment used by them, as proposed by Horwitt, Cowgill and Mendel (1936), is more efficient in decreasing nitrogen content than the pancreatin-sodium chloride used by Williams and Olmsted. The Horwitt, Cowgill and Mendel method was therefore chosen for the enzymatic treatment.

In the Williams and Olmsted method, both 68 and 72 percent sulfuric acid digestions were used. It was thought that the more dilute acid might cause less charring of the sugars, thus giving a lower lignin yield. Table I shows that the opposite is true, the reason, perhaps, being that the cellulose was not completely dissolved by the more dilute acid. The data show that the Davis and Miller method gives consistently lower lignin values than does that of Williams and Olmsted. These differences might be explained by the low results on hemicelluloses as determined on the lignin filtrate by the Shaffer-Somogyi (1933) non-fermentable reducing sugar method, relative to the total content of hemicelluloses of the indigestible residue (calculated from the furfural yield as determined by the Hallsworth (1939) procedure.) This procedure was followed since the filtrate from the strong sulfuric acid treatment in the Davis and Miller method cannot be satisfactorily analyzed for pentoses by the reducing sugar method. Table II shows that most of the pentoses in the indigestible residue are extracted by the 5 percent H_2SO_4 treatment. It also shows that the pentose results as de-

termined by the reducing sugar methods are nearly identical with those determined by calculation from furfural yield.

TABLE I

Comparative Results of Lignin Determinations by Olmsted-Williams and Davis-Miller Methods, and the Effect of the Former on Pentose Yield.

Material	Lignin found			Hemicellulose found		
	Olmsted-Williams		Davis-	Olmsted-Williams		From
	68% acid	72% acid	Miller	68% acid	72% acid	furfural yield
	mg/g	*mg/g*	*mg/g*	*mg/g*	*mg/g*	*mg/g*
Little bluestem	293.9	254.6	220.5	126.4	126.4	231.0
Sand paspalum	211.6	190.7	144.8	121.2		147.2
Hairy grama	251.0	191.5	155.0	91.2	94.7	162.5
Blowout grass	267.3		204.0	129.7	129.7	204.0
Buffalo grass	232.8	174.8	140.0	120.3	125.0	191.3

TABLE II

Comparative Results of Pentose Determinations[1] by Reducing Sugar Method and Furfural Method, After Separation of Pentoses by the Davis-Miller 5 Percent H_2SO_4 Treatment

Method	Pentoses in filtrate	Pentoses in residue	Total pentoses
	mg/g	*mg/g*	*mg/g*
Reducing sugars	215.5	83.5	299.0
Furfural yield	226.7	82.7	309.4

[1]On indigestible residue from sand bluestem grass.

CONCLUSIONS

The apparent lignin content as determined by the Davis and Miller method is consistently lower than that determined by the Williams and Olmsted method. The indications are that the higher lignin values obtained in the latter are due partly to the presence of hemicelluloses in the strong acid digestion mixture.

LITERATURE CITED

Crampton, E. W. and Maynard, L. A. 1938. The relation of cellulose and lignin content to the nutritive value of animal feeds. J. Nutrition 15: 383-395.

Davis, R. E. and Charles O. Miller. 1939. Partition of the less easily digested carbohydrate complex of forages. J. Ind. Eng. Chem., Anal. Ed. 11: 651-2.

Hallsworth, E. G. 1939. The analysis of the fibrous and carbohydrate constituents of feeding stuffs. I. The furfuraldehyde-yielding constituents. J. Soc. Chem. Ind. 58: 357-63.

Hilpert, R. S. and E. Littman. 1935. Lignin determination at low temperatures and the complete hydrolysis of straw. Ber. 68B: 16-18.

Horwitt, M. K., G. R. Cowgill and L. B. Mendel. 1936. The availability of proteins and inorganic salts of the green leaf. J. Nutrition 12: 237-254.

Norman, A. G. 1936. The composition of forage crops. I. Rye grass. Biochem. J. 30: 1354-1362.

Norman, A. G. 1937. Determination of lignin. III. Acid pretreatment and the effect of the presence of nitrogenous substances. Biochem. J. 31: 1567-78.

Norman, A. G. and S. H. Jenkins. 1934. The determination of lignin: I. Errors introduced by the presence of certain carbohydrates. Biochem. J. 30: 1354-1362.

Shaffer, Phillip A. and Michael Somogyi. 1933. Copper-iodometric reagents for sugar determination. J. Biol. Chem. 100: 695-713.

Williams, Ray D. and W. H. Olmsted. 1935. A biochemical method for determining indigestible residue (crude fiber) in feces: lignin, cellulose, and non-water-soluble hemicelluloses. J. Biol. Chem. 108: 653-666.