CVIII. A NEW METHOD FOR THE DETERMINATION OF CELLULOSE, BASED UPON OBSERVATIONS ON THE REMOVAL OF LIGNIN AND OTHER ENCRUSTING MATERIALS.

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IN the analysis of plant materials and woods, one of the chief constituents to be determined is cellulose, which is important since it forms the structural framework of the tissue. The determination of cellulose, however, is for various reasons not always satisfactory. The methods of determination of cellulose in general use do not all give the same product; consequently the results obtained are not always directly comparable. The method selected may be dictated by the purpose for which the cellulose is required. As it occurs naturally, cellulose is always found in the closest association with other constituents of the cell-wall, and these have, by various treatments, to be removed. Originally it was held that a number of distinct compound celluloses existed, *e.g.* lignocelluloses, pectocelluloses, *etc.* [Cross and Bevan, 1895], but the more recent view, which is generally accepted, is that all natural celluloses contain a certain fraction which is identical chemically. This fraction, typified by pure cotton cellulose, will from different sources give identical X-ray diagrams, comparable yields of cellobiose octa-acetate, and solely glucose on hydrolysis. It may therefore be regarded as "true" cellulose or "pure" cellulose in the rigid chemical sense. But cellulose as usually isolated does not yield only glucose on hydrolysis. In the case of hardwoods, cereal straws and certain fibre plants, xylose also is obtained, while in the case of softwoods mannose may be found in addition. These sugars are derived from polysaccharide material very intimately associated with the "pure" cellulose itself, and held either by adsorption or secondary valency forces. The association is so close that these polysaccharides may only be completely removed intact by the most violent treatment with alkali. Mild acid hydrolysis on the other hand removes them rapidly. These associated polysaccharides, which possess the general properties usually described for the hemicelluloses, have been termed by Hawley and Norman [1932] "cellulosans" and will be referred to as such in this paper. Development studies on the barley plant [Norman, 1933] show that the cellulosans are laid down with the "true" cellulose from the earliest stages and are not produced as the result of changes during lignification or senescence. The inference from this and other work is that the cellulosans must be regarded as forming a normal integral part of the cellulosic fabric of the plant, *i.e.* natural celluloses consist of "true" cellulose *plus* associated cellulosan. These two substances together will be taken in this paper to be covered by the word "cellulose."

As ordinarily found, natural cellulose is associated or encrusted with lignin and hemicelluloses of a polyuronide nature. Recent work on the molecular anatomy of "true" cellulose, as revealed by X-ray studies, makes it unlikely that primary valency linkages are involved in the association of lignin and cellulose, and physical theories of adsorption are usually proposed. Methods of cellulose determination therefore involve the removal of this lignin and encrusting hemicelluloses. The requirements of an ideal method are that lignin and polyuronide hemicelluloses should be rapidly and completely removed without affecting the integrity of the natural cellulose. It is in this last respect that a number of existing methods fail, inasmuch as they involve some treatment which attacks the less resistant cellulosan fraction.

The effect of pre-treatment on the yield of cellulose.

The first and by far the most important method is the chlorination process of Cross and Bevan, in which, by exposure to gaseous chlorine, a lignin-halogen derivative is formed and subsequently dissolved out together with the hemicelluloses by boiling with neutral sulphite. Alternate chlorination and boiling with sulphite have to be continued till, on addition of the sulphite, there is no red colour reaction indicative of lignin. In the case of many woods and fibres some three or four brief chlorinations are sufficient, but with the limited number of woods used in this work the number was considerably higher. Cross and Bevan [1895] originally proposed an alkaline pre-treatment of the material before chlorination, and boiled for 20 minutes with 1 % NaOH. Wood chemists in their official methods have, however, dispensed with this pre-treatment on the ground that the yield of cellulose is lowered thereby, as claimed by Renker [1910]. In the case of certain non-timber materials, as, for instance, cereal straws, chlorination without pre-treatment is an extremely tedious operation, in that as many as 9 or 10 exposures to chlorine and subsequent extractions with sulphite may be required, during which the cellulose becomes gelatinous and difficult to handle. In these cases an alkaline boiling is of great advantage, since the number of chlorinations then necessary is reduced to 2 or 3. Dorée [1933], discussing this point, advocates boiling for 5 minutes with 1 % NaOH on the grounds that it enormously facilitates subsequent delignification. It has been claimed that in the case of certain woods a lignin-free cellulose product is not attainable without prior alkaline treatment [*cf.* Strong, 1928]. Norman [1929], in work on the decomposition of cereal straws, employed the original Cross and Bevan procedure of boiling for 20 minutes with 1 % NaOH. There is little doubt, however, that advantageous as is this pre-treatment in expediting the removal of lignin, it is objectionable in that the cellulosan fraction may be partly attacked and suffer loss. Hawley and Norman [1932] drew attention to this on theoretical grounds and some experimental evidence on the point is given in Table I. Samples of three cereal straws were extracted for 20 minutes with boiling 1 % NaOH, filtered and washed thoroughly with hot water and a little dilute acetic acid. After drying and grinding, cellulose was determined by the Cross and Bevan method without alkaline pre-treatment. Total furfuraldehyde, lignin and furfuraldehyde due to cellulosan were also determined.

The effect of the alkaline pre-treatment on the cellulose yield is seen, as expected, to be a depressant one, the figures after treatment being less by the amount of cellulosan extracted. A measure of this is obtained from the figures for furfuraldehyde from the cellulose products, inasmuch as the cellulosan in the case of these three straws is a xylan. That subsequent delignification is

Table I. *Effect on cellulose determination of pre-treatment of straws with alkali.*
(Each straw boiled for 20 minutes with 1 % NaOH.)

All results expressed on the basis of 100 g. original dry straw.

Straw	Residue after extrac- tion	Cellu- lose after extrac- tion*	Furfurald. from cellu- lose after extrac- tion	Cellu- lose on original straw	Furfurald. from cellu- lose on original straw	Ap- parent lignin on original straw	Ap- parent lignin after extrac- tion	Total furfur- ald. on original straw	Total furfurald. after extrac- tion	Non- cellu- losic furfur- ald. on original straw	Non- cellu- losic furfurald. after extrac- tion
Oats	52.00	48.7	7.09	53.4	9.30	19.99	5.14 (74.30)	15.86	7.99 (49.60)	6.53	0.90 (86.20)
Wheat	62.52	53.7	9.55	55.2	9.96	20.93	6.02 (71.20)	16.64	10.58 (36.40)	6.68	1.03 (84.60)
Rye	66.31	57.2	10.18	60.2	11.44	21.56	6.97 (67.70)	14.80	12.05 (18.60)	3.36	1.87 (44.20)

* Cellulose determined by Cross and Bevan method without pre-treatment.

Figures in brackets represent percentage removed by pre-treatment with alkali.

facilitated is not surprising since the alkali treatment itself removes approximately 70 % of the total lignin of each straw. In addition a considerable proportion of the non-cellulosic furfuraldehyde-yielding constituents—the polyuronides—is removed by the pre-treatment.

Because of the difficulties mentioned above in determining cellulose in cereal straws by the Cross and Bevan procedure, Jenkins [1930] proposed a method involving the use of hypochlorite. Before submitting the straw to this reagent, however, it is boiled alternately for 5 minutes each with 1 % NaOH and 1 % HCl, these treatments being repeated twice. Jenkins [1930] quotes figures to show that the method gives results definitely lower than does Cross and Bevan's, but that this difference is due to loss of cellulosan rather than attack on "true" cellulose, which is identical in both cases. Unfortunately the figures were compared against the chlorination method with an alkaline pre-treatment. Had this been omitted, it would have been seen that the loss of cellulosan was great, and no doubt due to the hydrolysing effect of the acid boiling. To demonstrate in detail the losses occurring in each stage of the procedure advocated by Jenkins [1930] a large sample of straw was put through the process and analyses were made at each stage. By the Jenkins method the straw gave a "cellulose" figure of 39.56 %. The results are given in Table II.

Table II. *Analysis of effects of pre-treatments in Jenkins's cellulose method, using oat straw.*

All results expressed on basis of 100 g. original straw.

Treatment	Residue	Cellulose *	Furfurald. on cellulose	Total furfurald.	Non- cellulosic furfurald.	Apparent lignin †
None	—	53.5	9.2	15.9	6.7	19.9
Extracted twice with 1 % NaOH (5 mins.)	49.9	42.2 (21.2)	6.2 (33.2)	8.3	2.1 (68.7)	4.4 (77.9)
Extracted twice with 1 % HCl (5 mins.)	62.2	43.3 (19.2)	4.9 (47.2)	8.3	3.4 (49.2)	13.5 (32.2)
Extracted twice with alkali and acid (5 mins. each)	41.6	37.8 (29.3)	4.4 (51.9)	5.1	0.7 (89.5)	4.0 (79.9)

* By method described later in this paper.

† By 72 % sulphuric acid method.

Figures in brackets represent the percentage loss due to the treatment.

Although the individual treatments are brief the hydrolysing and extracting effects produced by them are very considerable. Alkali extracts more than does acid, mainly by reason of the extra lignin which it removes. The total furfuraldehyde yields of both acid- and alkali-extracted straw are identical but made up in rather a different way. The acid extraction apparently removes by hydrolysis over 45 % of the cellulosan, and alkali by solution less than 35 %, while on the other hand the effects of the two treatments on the non-cellulosic furfuraldehyde-yielding constituents are in the opposite direction. The apparent loss of lignin on brief treatment with acid was quite unexpected and will be referred to later. The combined effect of alkali and acid is a very drastic extraction of the straw, which loses nearly 70 % of its total furfuraldehyde-yielding groups and 80 % of its apparent lignin. The most serious result from the point of view of the subsequent determination of cellulose is that, by calculation from the furfuraldehyde in the cellulose, nearly 50 % of the cellulosan is removed. Pure cellulose, as typified by a high grade cotton, was shown by Jenkins [1930] to be unaffected by this treatment. The result of the subsequent three hypochlorite treatments is evidently very slight in comparison with that of the pre-treatments, for in this case the residue after the latter was 41.6 %, which was reduced to 39.56 % by the hypochlorite. Because of this slight effect of hypochlorite it was thought to be worth while to determine the lignin content of celluloses prepared by the Jenkins procedure. Some figures for straws are given in Table III, together with those for the Cross and Bevan product from the same material.

Table III. *Comparison of cellulose products obtained by Jenkins's method with those given by the Cross and Bevan procedure without pre-treatment.*

Straw	Jenkins cellulose in 100 g. straw	Furfurald. in 100 g. Jenkins cellulose	Apparent lignin in 100 g. Jenkins cellulose	C. and B. cellulose in 100 g. straw	Furfurald. in 100 g. C. and B. cellulose	Apparent lignin in 100 g. C. and B. cellulose
Oats A	41.9	9.1	4.8	53.5	16.8	3.0
Wheat A	47.5	9.9	7.8	55.2	17.7	3.1
Wheat B	45.1	10.4	4.6	56.7	18.3	2.9
Barley	35.2	8.9	3.9	47.7	—	3.3
Rye	51.9	10.9	6.6	60.2	19.0	2.4

Apart from the serious losses of cellulosan, to which attention has been directed above, it will be seen that the lignin figures given by celluloses prepared by the Jenkins procedure are hardly satisfactory. Even those obtained on the Cross and Bevan products are not as low as expected, an observation which will be dealt with in more detail below.

Therefore, although the method proposed by Jenkins [1930] may be suitable as a rapid and arbitrary procedure for determining a certain fraction of straws, a fraction not dissimilar from that given by the old-established crude fibre determinations, it cannot be regarded as providing a real indication of the cellulosic fabric of the material. It is faulty in two respects; firstly that the cellulosan fraction is very heavily attacked, and secondly that the product is not completely delignified.

*The rôle of sulphite in the determination of cellulose
by a chlorination procedure.*

The chlorination methods of cellulose determination, as previously stated, depend on alternate exposures to gaseous chlorine and extraction with boiling sodium sulphite in which the lignin-chlorine complex is soluble. At the same

time the hemicelluloses are removed by the boiling sulphite. It seemed to be of interest therefore to determine to what extent the chlorination itself is important. Samples of oat straw were given 1, 3 and 6 treatments with boiling sulphite for 20 minutes and the residues analysed for lignin and total furfuraldehyde. At the same time three other samples were given 1, 3 and 6 gaseous chlorinations for 10 minutes, each followed by boiling with sulphite for 20 minutes. The results are given in Table IV. Six neutral sulphite

Table IV. *Effect on oat straw of sulphite boils with and without chlorination.*

Treatment	Residue	Furfurald. on residue	Apparent lignin on residue	Furfurald. on original straw basis	Apparent lignin on original straw basis
1 chlorination*	73.4	19.2	12.2	14.1 (11.2)	8.9 (55.2)
1 sulphite boiling					
1 sulphite boiling	75.6	20.4	14.8	15.4 (2.8)	11.2 (43.8)
3 chlorinations	58.2	16.4	5.7	9.5 (39.9)	3.3 (83.4)
3 sulphite boilings					
3 sulphite boilings	68.4	19.6	12.5	13.4 (15.5)	8.5 (57.2)
6 chlorinations	52.2	17.2	4.4	9.0 (43.5)	2.3 (88.5)
6 sulphite boilings†					
6 sulphite boilings	62.7	19.7	8.5	12.4 (22.2)	5.2 (74.0)

* Chlorination by gaseous Cl—10 mins., 3 % sulphite boiling—20 mins.

† Still gave sulphite reaction for lignin.

Figures in brackets give percentage loss by treatment.

treatments alone remove 74 % of the lignin but only 22 % of the total furfuraldehyde-yielding constituents. The effect of the chlorination is greatly to expedite the removal of lignin, since at the end of the third chlorination as much as 83 % has been extracted. The chlorine however also has a marked effect on the removal of hemicelluloses, as indicated by the furfuraldehyde figures. Three chlorinations lower the furfuraldehyde yield by 40 % while three sulphite treatments alone only remove 15 %. This effect of chlorine in assisting in the removal of the hemicelluloses was unexpected and has not been commented upon before. It seems that the chlorination is at least as important in this respect as in removing lignin. Its mode of action is not understood at present, but will be the subject of further investigation. Neutral sulphite without chlorination has been proposed as a method of delignification of pulps but has not been found satisfactory, since high pressures and concentrations are necessary to obtain a satisfactory product [Rawling and Staidl, 1925].

Proposed new method for the determination of cellulose.

In view of the many chlorinations to which it is necessary to subject a cereal straw in order to obtain satisfactory delignification without pre-treatment, an attempt was made to devise a method more suited to this type of material. Jenkins [1930] observed that lignin could be removed in part by the use of sodium hypochlorite. It was found, however, that the delignifying effect could be enhanced by following the hypochlorite treatment by boiling with sodium sulphite, just as is done in the Cross and Bevan procedure. No colour reaction with sulphite, such as is given after treatment with chlorine gas, is observed.

The mode of action of the hypochlorite is not clear, and it can only be supposed that a soluble additive compound with the whole lignin molecule is obtained. After preliminary boiling with water, about 2 g. straw were allowed to stand for 15 minutes in 100 cc. water to which had been added 5 cc. hypochlorite containing approximately 15 % available chlorine¹. The straw was then filtered off on cloth and boiled for 20 minutes with 100 cc. 3 % sodium sulphite. This process was carried out one, two, three, four and six times on a sample of oat straw, with the results given below. Though the products after 4 and 6 hypo-

Table V. *Effect on oat straw of neutral hypochlorite treatments followed by sulphite boiling.*

Procedure	Cellulosic product	Apparent lignin % on cellulose
1 hypochlorite treatment	69.92	14.8
2 hypochlorite treatments	63.24	9.2
3 "	54.80	9.5
4 "	54.04	6.9
6 "	53.66	4.7
C. and B. 9 chlorinations without pre-treatment	53.99	3.1

chlorite treatments were excellent in appearance and retained the original structure they did not compare favourably in lignin content with those obtained by the Cross and Bevan procedure. Moreover, when exposed to gaseous chlorine, the product after 6 neutral hypochlorite treatments gave an intense purple reaction for lignin on addition of sulphite. Further, on subjecting different straws to 4 hypochlorite treatments, the effectiveness of the treatment was found to vary considerably, as will be seen in Table VI.

Table VI. *Comparison of products of four neutral hypochlorite treatments with those obtained by the Cross and Bevan method.*

Straw	Hypochlorite method		Cross and Bevan method	
	Cellulose product	Lignin on product	Cellulose product	Lignin on product
Oats A	54.0	6.9	53.4	3.1
Oats B	53.1	4.3	53.5	3.0
Wheat A	56.2	5.2	55.2	3.1
Wheat B	59.6	7.4	56.7	2.9
Barley	48.3	6.7	47.7	3.3
Rye	65.6	9.1	60.2	2.4

¹ An excellent and inexpensive solution of sodium hypochlorite may be obtained from Messrs Laporte of Luton. It contains hypochlorite equivalent to 15–17 % available chlorine, together with about 14 % NaCl, 1 % NaOH and 1 % Na₂CO₃. The strength of the hypochlorite may readily be determined by titration with thiosulphate in the presence of KI and H₂SO₄. The available chlorine or chlorine liberated on acidification represents twice the amount actually present as NaOCl, as shown by the equations

Owing to the presence of NaCl in the solution the same relationship holds on acidification with sulphuric or any other strong acid.

The equations given above have been verified experimentally.

In the absence of any easy and reliable test for lignin, clearly a method which involved a different number of treatments with different materials could not be very satisfactory. Accordingly it was decided to introduce a treatment with free chlorine so that the sulphite colour reaction for lignin might be employed and in the hope that the lignin might more completely be removed. The free chlorine was obtained by adding to hypochlorite an amount of sulphuric acid somewhat in excess of the equivalent so that hypochlorous acid should not be present to any extent. When cold sulphite is added to the straw from this acid hypochlorite treatment, if lignin is still present, a red purple colour develops just as if exposed to chlorine gas. In straws which have previously undergone a treatment with neutral hypochlorite and sulphite, the colour is more definitely purple than the red-brown colour usually obtained after chlorine gas. This suggests that the neutral hypochlorite removes some material or some portion of the lignin masking the purplish tint. Many experiments were carried out to determine the most satisfactory number combination of neutral¹ and acid chlorination treatments. To be practical it was considered that the number ought not in normal circumstances to exceed five, and accordingly the estimations were stopped at that point whether or not a sulphite reaction for lignin was still given. The results of some of the combinations of neutral and acid hypochlorite treatments are shown in Table VII.

Table VII. *Comparison of results of various combinations of neutral and acid hypochlorite treatment of oat straw.*

Treatment*	Cellulose on straw	Apparent lignin on cellulose	Lignin reaction at end
C. and B. 9 chlorinations	53.4	3.1	None
A.A.A.A.A. (2 mins. A.)	55.6	3.6	None
N.N.A.A.A. (2 mins. A.)	54.1	3.7	None
A.N.A.N.A. (2 mins. A.)	55.5	3.9	Slight
N.N.N.A.A. (2 mins. A.)	56.1	3.5	Definite
N.N.A.N.A. (2 mins. A.)	54.7	3.8	Trace
A.N.N.A.A. (2 mins. A.)	56.0	3.7	Strong
A.A.A.A.A. (15 mins. A.)	54.8	3.4	Slight
N.N.A.A.A. (15 mins. A.)	53.6	3.1	None
N.N.A.A.A. (5 mins. A.)	55.0	3.3	Slight
N.N.A.A.A.G. (5 mins. A.)	53.1	3.0	None
N.N.A.A.A. (10 mins. A.)	53.5	3.5	None

* N. 5 cc. NaOCl (15 % avail. Cl.) to 100 cc. water for 5 minutes, filtered and followed by sulphite boiling for 10 minutes.

A. 5 cc. NaOCl (3 % avail. Cl.) to 100 cc. water + 2 cc. 20 % H₂SO₄, filtered and followed by sulphite boiling for 10 minutes.

G. Exposure to chlorine gas for 5 minutes.

N.' As N. but for 10 minutes.

Chlorination with acid hypochlorite under similar conditions was found to be harmless to pure cellulose. Finely ground filter-paper given three such treatments for 5 minutes followed each time by boiling for 10 minutes with sulphite suffered a loss of only 2.7 %. Controls which were given three sulphite boilings without chlorinations lost precisely the same amount.

Arising from Table VII, the following method was adopted and found to be applicable not only to straws but also to woods. The material should be in a

¹ The treatments are spoken of as neutral and acid respectively for the sake of clarity. The neutral treatments are in fact faintly alkaline by reason of the free alkali in the sodium hypochlorite, necessary for stability.

uniform and fine state of division; in the case of woods 60–80 mesh appears to be most suitable, while in the case of straws 40–60 seems preferable. Woods must be given an alcohol-benzene extraction but this is unnecessary with cereal straws. About 2 g. material are brought to the boil in 100 cc. 3 % sulphite and filtered off on fine smooth poplin stretched over a Büchner funnel about 6 cm. in diameter and closely fastened either in a ring or with tight rubber bands. This preliminary sulphite boiling is advantageous not only in ensuring thorough wetting of the material but also in that a certain amount of lignin is removed, as shown in Table IV. Furthermore straws boiled with sulphite often filter much more readily than if treated only with water. The filtered material is then removed to a beaker by means of a spatula and jet from a wash-bottle. After making up the filtered straw to 100 cc., 5 cc. sodium hypochlorite (approx. 15 % available chlorine) are added and allowed to stand for 10 minutes. The material is filtered off, again transferred to a beaker and made up to 50 cc. with water; 50 cc. 6 % sodium sulphite are added, and the whole boiled for 20 minutes. It is usually convenient to bring to the boil over a flame and then to stand the beaker in a boiling water-bath, as bumping is frequently serious in the later stages. The treatment with hypochlorite is repeated a second time and followed by a sulphite boiling as before. The material, which by this time is much lighter in colour, is again suspended in 100 cc. water, and 5 cc. dilute hypochlorite (3 % available chlorine) are added, together with 2 cc. 20 % sulphuric acid. Chlorine is evolved and the material frequently turns yellow, just as it does in the Cross and Bevan process with gaseous chlorine. After standing for 10 minutes out of direct sunlight it is filtered, made up to 50 cc. and sulphite added as before. An intense purple coloration is given indicating the presence of lignin. This colour disappears on heating and the solution then becomes brown. The treatments with acid hypochlorite are continued as long as a positive reaction for lignin is given on addition of sulphite. In the case of some straws two acid treatments are sufficient. The process should not be continued longer than is necessary as indicated by this reaction. Sulphite is removed by suspension in 200 cc. or so of hot water, and after thorough washing the cellulose product is transferred to a Gooch crucible containing a small circle of cloth. It is dried to constant weight at 100°.

Chlorination under these conditions is without serious effect on pure cellulose. Samples of Whatman No. 1 filter-paper, finely ground, were given in duplicate the treatment described above but prolonged to the extent of 5, 7 and 10 acid chlorinations to determine the effect, if any, of gross over-chlorination. The recoveries from 1 g. were practically identical and equal to the amount obtained after 6 alternate cold and boiling-water extractions. The latter treatment represents water-soluble material *plus* mechanical losses in handling. These results are given in Table VIII.

Table VIII. *Effect of over-chlorination on pure filter-paper.*

Treatment	Amount recovered from 1 g. cellulose
2 N. 3 A.*	0.958
2 N. 5 A.	0.951
2 N. 7 A.	0.956
2 N. 10 A.	0.952
6 BW. 5 CW.†	0.952
Boiled in water 2 hours	0.991

* Times and concentrations as given in Table VII.

† Boiled for 20 minutes with water, filtered and allowed to stand in cold water for 10 minutes.

Table IX shows results obtained for certain straws and woods by this method, compared with the figures obtained by the Cross and Bevan procedure without pre-treatment. The cellulose figures are the averages of several determinations, while lignin and furfuraldehyde estimations were carried out only in duplicate. From this evidence it is claimed that the method gives results strictly comparable with those of the Cross and Bevan determination without pre-treatment, and that the product represents as closely as is possible the natural cellulosic tissue of the plant freed from encrusting substances. It possesses certain general advantages in that it dispenses with the use of chlorine gas and needs no permanent apparatus. With a sufficiently large water-bath to take the sulphite extractions after boiling, one worker can carry on as many as 16 determinations and, unless the material is especially resistant, carry them all through to completion in the course of a day. For cereal straws the method is preferable, since the number of chlorinations necessary is lessened, and the product does not become gelatinous or difficult to handle.

Table IX. *Comparison of results obtained on straws and woods by the Cross and Bevan method without pre-treatment and by the new method.*

Material Straws and woods	Cross and Bevan method				New method			
	No. of chlorina- tions	Cellulose %	Furfurald. on cellulose %	Apparent lignin on cellulose %	No. of chlorina- tions*	Cellulose %	Furfurald. on cellulose %	Apparent lignin on cellulose %
Oats A	7	53.4	17.4	3.1	2 N. 3 A.	53.5	17.2	3.5
Oats B	7	53.5	16.8	3.0	2 N. 3 A.	52.5	17.0	2.6
Wheat A	7	55.2	17.7	3.1	2 N. 3 A.	55.5	17.6	1.6
Wheat B	6	56.7	18.3	2.9	2 N. 3 A.	56.3	18.2	2.4
Rye	7	60.2	19.0	2.4	2 N. 4 A.	59.8	18.7	2.4
Oak (Danzig)†	10	53.2	18.1	0.3	2 N. 5 A.	53.9	18.2	0.7
Beech†	8	58.8	15.5	1.0	2 N. 5 A.	59.4	15.5	2.1
Scotch Pine†	8	60.2	5.2	0.8	2 N. 5 A.	60.8	5.5	0.2
Sitka Spruce†	6	66.8	3.3	0.2	2 N. 5 A.	65.9	3.2	0.1

* N. Neutral hypochlorite treatment. A. Acid hypochlorite treatment.

† Results expressed on material extracted with alcohol-benzene.

The method is easily applicable to large scale preparations of natural celluloses, and very satisfactory products have been obtained from a number of materials, both woods and straws. To obtain any quantity of cellulose by the Cross and Bevan procedure is a slow and laborious business in that the amount which can be chlorinated at any one time is limited. By the new method 200-300 g. can be treated at a time.

The determination of lignin in cellulose products.

One of the vital requirements in a method of determination of cellulose is, of course, that lignin be completely removed. Hawley and Wise [1926] state that small amounts of lignin are often tenaciously retained by the Cross and Bevan cellulose product, yet it is surprising that cellulose methods have rarely been subjected to this ultimate test, namely the lignin content of the product. Ritter and Fleck [1924] compare figures for lignin in celluloses treated with chlorine for long and short periods. They show that with the longer treatments the lignin remaining is only slightly lessened, but they fail to comment on the significance of the lignin figures themselves. Chlorinations were continued till no colour was given on addition of sulphite, but even so the lignin content varied from 1.0 to 1.5 %. In this work a lignin determination was carried out on every cellulose product, since the appearance is not a satisfactory criterion.

Preparations of excellent colour may nevertheless contain appreciable quantities of lignin. This is very true of products obtained by the Jenkins method in which delignification is incomplete. In Table IX is given the lignin content of products obtained from straw by both the Cross and Bevan and the new procedure, continued until no colour is given with sulphite. The method adopted was to add to the cellulosic material 20 cc. 72 % sulphuric acid, and after repeated stirring to allow the whole to stand overnight in a cool place, the temperature not being allowed to exceed 20°. After dilution with 250 cc. distilled water, the solution was boiled for half an hour, allowed to settle and filtered. The longer period with 72 % acid and short hydrolysis at an acid concentration of about 9.5 % is preferred to the present method advocated by the U.S. Forest Products Laboratory, which involves only a brief treatment with strong acid and 4 hours' hydrolysis after dilution to an acid concentration of 3 %. By the method adopted the Cross and Bevan celluloses from straws contained approximately 3 % lignin, and this figure could not be lowered appreciably by over-chlorination. Celluloses prepared by the new method were uniformly a little lower, but, even so, averaged about 2.5 %. Inasmuch as in both cases the wood celluloses were very considerably lower in lignin content it seemed that this high lignin figure might be apparent rather than real, and the reason for it inherent in the nature of the straw.

Recently a number of investigators [*e.g.* Wise and Fairbrother, 1931; Sherrard and Harris, 1932] have pointed out some of the defects of the 72 % sulphuric acid method for lignin, and some have made diametrically opposed recommendations as to the conditions under which the determination should be carried out. Ritter, Seborg and Mitchell [1932] suggest a brief treatment at 20°, while Peterson, Walde and Hixon [1932] prefer lower temperatures for a longer period and recommend that the reaction should be carried out in an ice-box. All are agreed that acid of this strength causes a certain amount of charring or caramelisation of the carbohydrate material, and that this may produce serious errors if the temperature rises above about 20°. Hawley and Wiertelak [1931] and Hawley and Harris [1932] have shown that a lignin-like material may be produced by exposure of cellulose or wood to a temperature of 135° for several days. Of the carbohydrate material concerned, the cellulosan, which is pentose in nature in the hardwoods and largely hexose in softwoods, suffers the greatest loss. Since, by a violent process of dehydration by heat, apparent lignin can be obtained in considerable amount from the cellulosans, it seemed possible by analogy that smaller quantities of some similar material might be formed by the action of 72 % sulphuric acid as used in the lignin determination. In some work, as yet unpublished, Norman has found that the cellulosans are readily removable by dilute acid hydrolysis. Lignin presumably is unaffected by dilute acid, since prolonged boiling in acid concentration of 4 % or more is generally accepted as a part of the estimation procedure. If, then, this theory is correct, the apparent lignin content of a cellulose from which the cellulosan has been removed by hydrolysis would be reduced as a result of the treatment. A sample of oat-straw cellulose prepared by the proposed new method had an apparent ash-free lignin content of 3.47 %. On boiling for 1 hour with 3 % H_2SO_4 , a treatment known to remove a part, but not all, of the cellulosan, the cellulose suffered a loss of 17.1 % and the product had then an apparent lignin content (on the basis of the original cellulose present) of 2.37 %. Similarly a sample of oat-straw cellulose, prepared by the Cross and Bevan process and containing 3.0 % apparent lignin before hydrolysis, gave under the same treatment a product containing only 2.23 %. The effect of the

removal of part of the cellulosan in both cases therefore was to lower the apparent lignin content by nearly a third. It seems clear that from the pentose material some product is being produced insoluble in boiling dilute acid and therefore determined as lignin. The 72 % sulphuric acid solution of the cellulose after removal of cellulosan was, incidentally, very markedly lighter in colour than that of the untreated material. The effect of hydrolyses with different strengths of acid for various times in lowering the apparent lignin content was next studied. Cellulose was prepared from wheat straw by the hypochlorite method, two neutral and three acid treatments being given. The product had an apparent lignin content of 2.4 %. 1.5 g. samples were hydrolysed under several conditions, filtered, dried and weighed and the lignin content of the residues then determined. The results are given in Table X. Treatment with

Table X. *Effect of hydrolysis of celluloses on the apparent lignin content of a wheat-straw cellulose containing 2.4 % lignin.*

H ₂ SO ₄ strength %	Time in hours	Loss %	Furfurald. in solution %	Apparent lignin %
1.0	$\frac{1}{2}$	15.5	5.52	2.2
3.0	1	23.6	—	1.9
3.0	2	24.8	—	2.1
3.0	3	25.8	10.44	1.8
5.0	1	25.2	—	1.7
5.0	2	27.2	10.52	1.9
10.0	3	35.3	12.36	1.6

10 % acid, which, of course, results in appreciable hydrolysis of the cellulose itself as well as complete removal of the cellulosans, lowered the apparent lignin figure by a third to 1.6 %. Since this is still an amount higher than that found for most woods, it seemed desirable to investigate the nature of this residual apparent lignin, and to attempt to decide whether it is true lignin or secondary material produced in the course of the reaction. If true lignin, then it ought, perhaps, to be removable by gross over-chlorination and repeated extractions, whereas, if secondary material, such violent treatments should leave the amount very little changed.

Wheat straw of apparent lignin content 20.3 % was treated in a series of 1.5 g. samples by the new method for cellulose determination. All the samples were given 2 neutral hypochlorite treatments, and then in pairs 1, 2, 3, 5 and 7 acid hypochlorite treatments respectively, each being followed by sulphite boiling. The apparent lignin was determined directly in one of the two duplicates and in the other after hydrolysis for 1 hour with 5 % H₂SO₄ to remove the cellulosan, as described above. The results are presented in Table XI. It will be seen that the apparent lignin content is appreciably lowered in all cases but the last by removal of the cellulosan. Even on gross over-chlorination to the extent of five extra acid hypochlorite treatments after the last trace of reaction with sulphite, the apparent lignin is quite appreciable, amounting to about 1.5 % on the original straw basis. Since it might have been supposed that these continued chlorinations would remove any residual lignin, this residue might be ascribed to material secondarily produced from the cellulose itself by charring or caramelisation. This view, however, is not supported by evidence from other materials. Results obtained with wood celluloses are of a considerably lower order, and both hardwood and softwood cellulose products have been obtained, as shown in Table IX, with an apparent lignin content as low as 0.1 to 0.5 % on the basis of the cellulose. Other hexosan material, such as

Table XI. *Removal of apparent lignin from wheat straw at successive stages of chlorination.*

All results expressed on original dry straw. (Apparent lignin = 20.3 %.)

Treatment	Colour reaction with sulphite	Product on chlorination %	Residue after hydrolysis with 5 % H_2SO_4 %	Apparent lignin in chlorination product	Apparent lignin in hydrolysis residue
—	—	—	57.23	—	15.2
2 N.	—	68.56	48.04	8.3	6.7
2 N. 1 A.	Strong	54.91	42.81	3.3	2.1
2 N. 2 A.	Trace	53.03	41.12	3.1	1.7
2 N. 3 A.	Nil	51.45	41.02	3.2	2.0
2 N. 5 A.	Nil	48.73	41.02	3.0	1.9
2 N. 7 A.	Nil	48.03	40.84	1.5	1.7

pure filter-paper or starch similarly yield only a trace of apparent lignin. It is not natural to suppose that straw cellulose is more susceptible to charring or caramelisation than other celluloses, since the magnitude of the apparent lignin figures is so different. The residue obtained, therefore, must be either lignin so tenaciously retained as to be unaffected by chlorination and sulphite extraction or some other acid-resistant constituent of unknown composition. This point will be tested further and an attempt made to characterise or extract this material by some such means as a modification of the glycol method of Hibbert and Rowley [1930].

Additional support for the view that from pentose material there is produced some material with the acid-resistant properties of lignin was obtained by carrying out that determination on filter-paper alone, filter-paper *plus* glucose and filter-paper *plus* xylose. 1 g. of filter-paper alone yielded 0.02 mg. apparent lignin, with the addition of an equal weight of glucose 0.03 mg. apparent lignin, but with the addition of only 0.25 g. xylose as much as 5.5 mg. apparent lignin.

Although somewhat outside the scope of this work the information obtained on the formation of apparent lignin from pentosan groupings may be of great importance in the ordinary determination of lignin in untreated materials. W. G. Campbell in a private communication has expressed the opinion that the hemicelluloses are a source of error in the lignin determination. It may therefore be necessary to modify the method by the introduction of a mild hydrolytic treatment for the removal of these constituents. In Table II is shown the effect of a brief extraction of oat straw with HCl, the apparent lignin content

Table XII. *Effect on apparent lignin content* of preliminary hydrolysis of straws and woods (5 % H_2SO_4 for 1 hour).*

Results expressed on 100 g. material.

Straw	Apparent lignin %	Loss on hydrolysis %	Apparent lignin after hydrolysis
Oats	18.4	42.5	12.6
Wheat	19.3	37.5	13.7
Barley	15.7	49.4	11.2
Beech†	22.8	26.7	19.2
Scotch pine†	24.1	23.6	23.7

* Determined by treatment for 16 hours with 72 % H_2SO_4 , dilution to 3 % and boiling for 2 hours. These figures therefore are directly comparable with those obtained by other workers.

† Results expressed on material extracted with alcohol-benzene.

falling from 19.9 to 13.5 %, a loss of no less than a third as a result of a treatment which would presumably not affect true lignin. Similarly some of the straws and woods employed were boiled for 1 hour with 5 % sulphuric acid and almost equally large losses in apparent lignin content noted, as shown in Table XII.

These great differences make it clear that the whole question of the determination of lignin by the 72 % sulphuric acid method must be critically re-examined, since, if the indications given here are correct, the quoted lignin figures for straws and hardwoods are too high as a result of secondarily produced material from the pentose groupings. Those for the softwoods, in view of the smaller content of pentose material, are probably more nearly accurate. This may be seen from the figures given for beech and Scotch pine in Table XII.

SUMMARY.

1. The celluloses or polysaccharide material very intimately associated with "pure" cellulose in nearly all natural celluloses should remain intact in any satisfactory method of cellulose estimation.

2. Alkaline pre-treatment, as often recommended, is objectionable in that a portion of the cellulose is removed, even though delignification is much assisted.

3. Jenkins's method of cellulose determination is unsatisfactory for two reasons. First, the preliminary treatments with alkali and acid alternately result in a very severe loss of cellulose, and second, the product is incompletely delignified by the subsequent treatments with neutral hypochlorite.

4. In the chlorination methods of cellulose determination the chlorine is at least as important in causing the solution of hemicelluloses during the subsequent treatment with boiling sulphite as in assisting in the removal of lignin. Sulphite alone results in extensive, but not complete, delignification without heavy loss of encrusting polyuronide hemicelluloses.

5. Neutral hypochlorite treatments followed by sulphite boilings will effect a fairly complete delignification of plant materials.

6. A new method is proposed for both straws and woods involving first two treatments with neutral hypochlorite and then three or more with acid hypochlorite, each followed by boiling with sodium sulphite. The results obtained are almost identical with those obtained by the Cross and Bevan method without pre-treatment. The proposed method is more rapid with straws in that it involves fewer treatments. It is readily applicable to large scale preparations.

7. The products from cereal straws given by both the Cross and Bevan and the new methods are not entirely free from lignin. The lignin figure obtained by the use of 72 % H_2SO_4 on such products is, however, not accurate, since appreciable quantities of apparent lignin are produced from the cellulose when pentose is nature. Upon removal of this fraction by acid hydrolysis the lignin figures are reduced by a quarter to a third, the residue being unaffected by over-chlorination. It must therefore be either true lignin very tenaciously held, or else some other resistant material of unknown composition.

8. In view of the production of apparent lignin from pentosan groupings by 72 % H_2SO_4 , the figures obtained for lignin on many natural materials may be unreliable. A brief preliminary acid hydrolysis of straws results in the lowering of the apparent lignin content by 25-30 %. Hardwoods similarly are likely to give figures which are too high.

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