

fere with alcoholic fermentation. In other words the manufacture of a vinegar of standard strength may be seriously affected by an admixture of acetified apple juice with juice of sound apples.

THE ESTIMATION OF CELLULOSE IN WOOD BY THE CHLORINATION METHOD.

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The chemical constitution of woody tissues presents a problem of great complexity. Any single sample of wood contains a number of complex compounds whose nature is but little understood. If all kinds of wood were chemically alike, or even if the wood from all parts of the same tree was identical, the study of woody tissue would be simplified. The older idea, especially the idea prevalent amongst botanists, was that the cell walls of woody tissues were composed of "cellulose" which became impregnated with "lignin." Later and more careful studies appear to have established the fact that the foundation of woody cell walls is not a simple cellulose, but a compound cellulose, to which the name lignocellulose, has been given. This lignocellulose may be split up into cellulose on the one hand, and the lignone group on the other. The cellulose which results from the cleavage differs to a marked extent from the normal type cellulose of the cotton fiber. The lignone complex appears to contain cyclical groups and to be related to the carbohydrates in no very definite fashion.

Besides the lignocellulose which makes up the major portion of wood, there are always present a number of different bodies which are apparently not chemically united to the lignocellulose. Amongst these substances two are prominent; the so-called wood gum, which may be extracted with cold dilute alkaline solutions, and the group of the ether soluble resins.

It was stated above that the lignocellulose might be split into the two constituent radicals, cellulose and lignone. This cleavage is not easily carried out quantitatively, inasmuch as the treatments which remove the lignone tend to attack the cellulose. The isolation of cellulose from wood is carried out on an enormous scale in the manufacture of paper pulp by the sulphite and soda processes. These methods are said to always involve some loss of cellulose, and unless carefully regulated the loss is likely to be great.

A number of methods have been elaborated for the estimation of the quantities of cellulose in vegetable cell walls. They are all tedious, and the cellulose obtained by several of them is apparently a variable and ill defined product. Cross and Bevan¹ have elaborated a method for the estimation of cellulose in the lignocellulose of jute fiber, a method which

¹ Cellulose: an Outline of the Chemistry of the Structural Elements of Plants. Cross and Bevan, London, 1895.

appears to yield concordant and valuable results. In their method the lignocellulose is treated with chlorine gas, giving rise to a lignone chloride which is soluble in hot alkaline sulphite solutions. They do not give the results of the systematic application of this method to cellulose estimations in wood, although they imply that it is applicable to such tissues.

Sherman¹, in his study of the insoluble carbohydrates of the wheat, estimated the amount of cellulose in the lignocellulose of the wheat kernel. After trying out the methods of Schulze (potassium chlorate and nitric acid,) Lange (fusion with caustic alkalies), and the chlorination method of Cross and Bevan, Sherman concludes that this last method gives the most exact estimate of the cellulose and yields cellulose of maximum purity.

Cross and Bevan give the following directions.² "About 5 grms. of the fibre weighed after drying at 100°—are (a) boiled for 30 minutes with a dilute solution of sodium hydrate (1 per cent. NaOH), which is kept at constant volume by addition of water. The fiber is well washed on a cloth or wire gauze filter, squeezed to remove excess of water, opened out, placed in a beaker, into which (b) a slow stream of washed chlorine gas is passed. Rapid reaction ensues, and the fiber changes in color, from brown to a bright golden yellow. To ensure complete conversion of the lignone, it is necessary to leave the fiber for some time (from 30-60 minutes) in the atmosphere of Cl gas. (c) The chlorinated fiber is removed, washed once or twice with water to remove hydrochloric acid, and placed in a 2 per cent. solution of sodium sulphite; the solution is gradually raised to the boiling point, a small quantity of caustic soda solution is added (0.2 per cent. NaOH calculated on the solution), and the boiling continued for 5 minutes. (d) The cellulose is now thrown upon a cloth filter and washed with hot water. It will be found to be almost pure *i.e.* white; but to remove the last residues of the non-cellulose, it may be bleached by immersion in a dilute solution of hypochlorite (0.1 per cent. NaOCl for a few minutes, or treated with dilute permanganate solution (0.1 per cent. KMnO₄). It is well washed from these oxidizing solutions, treated with sulphurous acid on the filter, well washed with water, squeezed, dried and weighed."

In another place these authors state that in applying this process to wood, it is necessary to reduce the wood "to the finest possible shavings." In giving directions for obtaining cellulose from pine wood³ they say that after treating the finely divided wood as in the above quoted passage, the chlorination must be repeated until pure cellulose is obtained.

It is, of course, impossible to follow the directions given above when

¹ Sherman: J. Am. Chem. Soc., 19, 291, 1897.

² Cellulose: p. 95.

³ *loc. cit.*: p. 244.

using finely ground wood, since the quantitative removal of such a material from a gauze or cloth filter, without the use of a stream of water, is practically impossible. After some experimenting we adopted the following method of procedure.

The wood was dried out at a low temperature, 60°-80°, and ground in a hand mill. The material was then sifted through a sieve having twenty-four meshes to the inch. The wood powder obtained in this way was dried at 100°-105° and weighed portions of about five grams each used for each determination. The sample, after being boiled in 200 cc. of 1 per cent. sodium hydroxide solution for a half hour, was filtered with suction on a small perforated porcelain plate in a five-inch funnel. In order to hold the plate firmly in the funnel during the filtration and the subsequent manipulation, we passed a piece of fairly stiff silver wire up through one of the holes in the center of the plate and securely fastened it by bending the end down. The silver wire extended down through the stem of the funnel, projecting a quarter of an inch beyond the end. By putting one or two slight bends in the wire it can be made to bear against the walls of the inside of the stem, and the filter plate is held firmly in place. When the ground wood is first poured on to such a plate it behaves much like asbestos, some running through and the rest matting down and forming a coherent layer. The portions running through are then poured back, and by careful manipulation no significant loss of fiber occurs. The wood which had been boiled in alkali was washed on the filter with a large volume of water. The matted fiber was loosened up with a sharp pointed glass rod and the rubber tube leading from a chlorine generator attached to the stem of the funnel. A slow stream of washed chlorine gas was passed up through the material, which was occasionally stirred to allow a thorough penetration of the gas.

After the chlorination the funnel was returned to the suction flask and the fiber washed to remove hydrochloric acid. For the boiling in sodium sulphite solution it was necessary to remove the material to a casserole. This may be readily accomplished by inverting the funnel over the casserole and pushing on the silver wire projecting from the stem. The main mass of the material drops out and the remainder is readily washed from the funnel and filter plate with the aid of 150 cc. of 2 per cent. sodium sulphite solution which has been previously measured into a wash bottle. After bringing the sulphite solution to a boil, 3 cc. of a 10 per cent. sodium hydroxide solution were added and the mixture boiled for five minutes. The fiber was again collected on the filter plate, washed with hot water until the washings were colorless, and the product again chlorinated, or, when desired, bleached in the funnel by the use of 0.1 per cent. potassium permanganate solution followed by sulphurous acid.

In order to conveniently weigh the final product it was transferred from the funnel to a beaker, with the aid of alcohol from a wash bottle, and then collected in a large, tared Gooch crucible, dried and weighed.

A single chlorination, even if prolonged for two hours, was insufficient to yield a white cellulose after permanganate bleaching. The greatest difficulty in the whole process lay in judging the end point. Finely ground wood is not homogenous, since there are always small pieces made up of cells from the summer wood of the annual rings, and these cells with their thick walls offer a marked resistance to reagents. It frequently happened that after a series of chlorinations and permanganate bleachings which reduced the great mass of the sample to white cellulose that there still remained particles which were yellow. Since treatment with chlorine gas will attack wood cellulose to some extent, it became questionable whether or not continued chlorination did not cause a loss of cellulose in the finer particles which introduced a greater error than that caused by stopping the treatment before the sample was uniformly white.

The lignocelluloses when immersed in a mixture of equivalent solutions of potassium ferricyanide and ferric chloride are stained blue with the fixation of both iron and cyanide groups. The gain in weight occurring in this reaction varies with the quantity of ferric ferricyanide solution which is used. Sherman found that with the same volumes and strengths of solutions, and the same time of immersion, the gain in weight was constant for a given sample. By applying the reaction to the cellulose obtained by the different methods, he obtained a criterion of their purity. Of the use of the reaction he says,¹ "the reaction with the ferricyanide solution seems of considerable value in testing the purity of celluloses. When the sample increases only slightly (about one per cent or less) in weight and is dyed only a bluish-green instead of a deep blue color, it is at least a very strong indication that lignin substances are not present in notable quantity."

In applying this reaction to the products obtained by us, we have followed Sherman's procedure: 10 cc. of 1.6 per cent. solution of ferric chloride were mixed with 10 cc. of a freshly prepared 3.3 per cent. solution of potassium ferricyanide; to this mixture 1 gm. of dry material was added and after standing one hour, with occasional stirring, the dyed fiber was collected on a Gooch filter, washed with water, dried, and weighed. In some cases the filtrate from the fiber was a deep green instead of the brownish-red color of the ferric ferricyanide solution, and it happened in a number of cases that a loss of weight followed the treatment, showing that soluble products were formed by the action of the ferricyanide solution on some of the constituents of the cellulose.

The results of the first series of estimations are given in Table I. After

¹ loc. cit.: p. 304.

weighing out the samples of the more resinous woods, they were extracted with ether before the alkaline boiling.

TABLE I.

Wood	Length of Chlorination	Appearance of Cellulose	Per cent. of Cellulose	Per cent. gain with ferricyanide reaction
Longleaf Pine—sapwood	2 chlor. of 1 hr. & ½ hr.	Cream color	53.5	
“ “ “	4 “ “ ½ hr. each	white	53.8	
“ “ “	2 “ “ “ “	Light cream	58.4	
“ “ heartwood*	3 “ ¾ hr., ¾ hr. & ½ hr.	(uneven) white	45.8	
“ “ “	3 “ “ “ “ “ “	Light cream	49.0	
“ “ “	3 “ “ “ “ “ “	Dark	52.8	
Loblolly pine—sapwood	3 “ ½ hr. each	“	60.8	
“ “ “	2 “ “ “	“	63.3	
“ “ “	3 “ ½ hr. & 1 of ¾ hr.	white	53.9	
“ “ “	2 “ “ “	“	54.5	
“ “ heartwood*	2 “ ¾ “ 1 “ ½ “	Light cream	49.6	3.80
“ “ “	3 “ “ “	“	48.1	2.69
Lodgepole pine—sapwood	2 “ “ “	“	59.4	6.71
“ “ “	2 “ “ “	“	58.8	4.18
“ “ heartwood	3 “ “ “ & 1 of ½ hr.	white	43.7	2.36
“ “ “	3 “ “ “ “ “ “	Very white	40.4	4.05
“ “ “	2 “ “ “	Cream color	49.5	-59
“ “ “	2 “ “ “	“	50.4	-3.77
Spruce-mixed heart & sap	2 “ ½ hr. & 1 of ¾ hr.	Light cream	59.6	3.94
“ “ “	2 “ “ “	Dark	63.8	6.07

*In boiling with Na_2SO_3 after the first chlorination of this sample the solution was made alkaline to 0.4 % instead of the usual 0.2%.

The results which are summarized in Table I show that the chlorination method is not easily controlled for quantitative estimations. In estimations of this sort it is not to be expected that the results will be concordant to small fractions of a per cent, but the variations observed seem rather too large even for proximate analyses. As stated above, the difficulty of judging the end point of the process was very great. The same treatment did not always yield the same kind of a product, nor show the same percentage of cellulose. If the chlorinations were continued until a perfectly white product was obtained, the percentages were not always alike and seemed to be too low. The results of the ferricyanide reaction did not appear to be of much help.

To assist in clearing up some of these points, we have carried out a series of systematic determinations on four different kinds of wood. Of each kind of wood four (five in the case of No. 1) samples of about five grams were taken. The first sample was boiled in alkaline solution as described above, chlorinated once for one hour, boiled in sulphite solution, treated with permanganate and sulphurous acid, washed and dried; the next sample was treated in precisely the same way except that it had two one hour chlorinations and the accompanying sulphite boilings; and so

on, each sample receiving one more chlorination than the one before it. In the case of Wood No. 1 the final washings were carried out in the funnel, as in the former experiments, with the other woods the final product was boiled for five minutes in a large volume of distilled water in order to ensure the removal of all reagents. The different kinds of wood, and each of the celluloses, were tested by the ferricyanide reaction. The results are summarized in Table II.

TABLE II
WOOD NO. 1: HEARTWOOD OF RED SPRUCE.

	Color	Per cent. of Cellulose	Per cent. gain by Ferricyanide Reaction
Wood.....	13.85
Cellulose by 1 chlorination	light brown	61.9	2.52
“ “ 2 “	light cream	58.5	0.11
“ “ 3 “	white	51.9	— 1.50
“ “ 4 “	“	49.1	— 1.55
“ “ 5 “	“	46.8	— 1.42

WOOD NO. 2: SAPWOOD OF RED SPRUCE.

Wood.....	9.51
Cellulose by 1 chlorination	light brown	63.5	6.69
“ “ 2 “	light cream	53.5	0.79
“ “ 3 “	white	51.3	— 1.36
“ “ 4 “	“	47.9	— 2.83

WOOD NO. 3: HEARTWOOD OF BALSAM FIR.

Wood.....	11.63
Cellulose by 1 chlorination	light brown	57.3	4.2
“ “ 2 “	cream	49.4	2.54
“ “ 3 “	white	44.1	2.02
“ “ 4 “	“	44.6	1.75

WOOD NO. 4: SAPWOOD OF LODGEPOLE PINE.

Wood.....	7.77
Cellulose by 1 chlorination	dark cream	54.6	1.36
“ “ 2 “	light cream	54.3	1.40
“ “ 3 “	white	52.3	0.87
“ “ 4 “	“	49.1	— .10

None of the woods here are very resistant, and in only one case, Balsam Fir, was trouble given by the presence of refractory particles which would not become perfectly white. In all cases when the fiber had received two chlorinations of one hour each, it bleached when again subjected to the action of chlorine gas, instead of turning to the orange-red characteristic of lignocelluloses. Cross and Bevan believe that this orange color with chlorine is a *characteristic* reaction. If that be true, then the complete removal of the lignone complex must take place early during the third chlorination, and the percentage of cellulose falls in between the values found with two and with three chlorinations. The ferricyanide reaction fails to give wholly satisfactory results. We have never been able to obtain a cellulose from wood which does not give

a blue-green color with this reagent. Why the deep green filtrates should be obtained from some of the tests, and a loss of weight should occur, is not apparent.

The final working out of the following satisfactory analytical procedure was greatly aided by the realization that the end point was indicated by the bleaching of the cellulose in chlorine, and by finding that satisfactory samples could be secured by the use of a wood-worker's rasp.

By holding the wood to be sampled in a vise, and rasping lightly, a finely fibrous sample is readily obtained. The best samples are prepared from green wood; when dry wood is to be sampled, it should be soaked or boiled until thoroughly softened before being rasped. The material is brought to an air dry condition, any large pieces which may perchance be present picked out with forceps, and the very fine particles removed by shaking the air dry sample on cheese cloth or a very fine sieve. The resulting uniform material is then dried at 98°-100°, and 5 gm. portions used for analysis.

The first chlorination should be for one hour; the length of subsequent chlorinations must be to some extent a matter of judgment. If the fiber takes on a very deep orange, showing that a large amount of the lignone is still present, the second chlorination should be for a half or even three quarters of an hour; if lighter colors appear, the time should be shorter. The wood of deciduous trees gives a far paler orange color with chlorine, than that of the coniferous species, and more readily yields a pure cellulose.

The rate of chlorine flow influences the length of exposure required. This is brought out in analyses 1 to 4 in Table III. In analyses 1 and 2 the chlorine was passed in slowly, the first chlorination was not complete, and a second treatment was required. In analysis 3, the gas was passed in more rapidly, and when the fiber was exposed to chlorine the second time it bleached at once. The final result is not influenced, provided the fiber is removed immediately after bleaching occurs. If the cellulose is not removed from the action of chlorine as soon as bleached, it is attacked and a significant loss occurs, as shown in analysis No. 4. When bleaching occurs, the funnel should be transferred to the filter flask, the cellulose treated with water containing a little sodium bisulfite to act as an "anti-chlor," then thoroughly washed, transferred to a large size Gooch crucible, and finally washed in the crucible with alcohol and ether. The first part of the drying should be at a gentle heat, ending with a temperature of 98-100°.

Several analyses are given in Table III, all of them satisfactory, except No. 4, where the chlorine was allowed to act on the bleached cellulose.

TABLE III.

No.	Material	Chlorinations	Per cent. of Cellulose
1	White oak, sapwood.....	1 hour, 20 minutes and to bleach	55.90
2	" " "	" " " " " "	56.34
3	" " "	" " and to bleach	56.40
4	" " "	" " " " " "	54.79
5	Red cedar, "	" " 30 minutes and to bleach	42.04
6	" " "	" " " " " "	41.51
7	" " heartwood.....	" " " " " "	42.52
8	" " "	" " " " " "	43.00
9	Red spruce "	" " 10 minutes and to bleach	58.95
10	" " "	" " " " " "	58.60
11	Cherry, sapwood.....	" " and to bleach	54.72
12	" " "	" " " " " "	54.43

The final products were all pure white, and none of them gave the lignin reaction with phloroglucinol and hydrochloric acid.

In conclusion, we desire to acknowledge our indebtedness to Mr. D. C. Parmelee for valuable assistance given us in the analytical work.

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NOTES.

On the Determination of Fluorine.—When fluorine is determined by the Berzelius¹ method the results vary and are considerably too low. Seven determinations gave 76 to 94 per cent. fluorine as calcium fluoride. I repeated the work of Seeman² but without being able to find where the loss occurred. Fusing the fluoride with sodium peroxide in a nickle crucible gave no better results. Fourteen determinations, gave 81.4 to 94 per cent. of calcium fluoride taken. I am led to believe that some of the fluorine is retained in the fusion residues as a complex fluoride³, probably in combination with silica.

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The Rapid Determination of Water in Butter. Aluminum Beaker Method.—In the November (1906) Journal,⁴ I described a method for the rapid determination of water in butter, of sufficient accuracy for factory purposes, which consisted merely in boiling the water off from a weighed portion of butter contained in a wide test-tube heated by the naked flame of an alcohol lamp. Very shortly thereafter I made a seemingly slight modification in the method, by adopting the use of an aluminum beaker in place of the test-tube—a mere change in utensil, but a change which so greatly improves the method, both by facili-

¹ Pogg. ann. 1, 169; Schweigg. Jsb., 16, 426; and Treadwell: Kurges Lehrbuch d. Analyt. Chem., Bd. II, 2te, Auf., p. 326.

² Z. Anal. Chem., 44, 369 (1905).

³ Z. Anal. Chem., 33, 505 (1904); Z. Anorg. Chem., 51, 168 (1906).

⁴ This Journal, v. 28, 1611.