The Use of Bromine in the Estimation of Cellulose in Plant Materials

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A number of methods are available for the quantitative estimation of cellulose in plant materials and have been reviewed by Dorée (1933). Almost all of them are based upon the original procedure of Cross & Bevan (1903), who used chlorine gas for the removal of lignin as 'lignone chloride' followed by hot sodium sulphite extraction. Since then Jenkins (1930) and Norman & Jenkins (1933) have proposed the use of chlorine in solution as sodium hypochlorite to obviate the inconvenience of using gaseous chlorine. The defects in the original Jenkins (1930) method were subsequently discussed by Norman & Jenkins, who developed a modified method which eliminated them. Chlorination in this method is effected by acidification of dilute hypochlorite solution and, since the gas is liberated in contact with the material, treatment is effective and sometimes reduces the number of chlorinations necessary.

Although the use of chlorine in solution is advantageous it is difficult to obtain and store sodium hypochlorite in the tropics owing to its instability. In order to obviate a return to the use of gaseous chlorine, it was thought that sodium hypobromite, which is more easily prepared than the hypochlorite, might prove equally useful in cellulose determination, or that, alternatively, nascent bromine might well be liberated in contact with the material by acidifying a mixed solution containing bromide and bromate. After a few tests the method seemed to work satisfactorily enough to warrant the working out of a detailed analytical procedure based on the principles laid down in the chlorination processes. Although Dorée (1933) and Norman (1937), two of the more recent authors on cellulose chemistry, make no reference to the use of bromine in cellulose determination, Schwalbe (1938) has referred to the application of bromine for this purpose by Hugo Müller in 1877 in the form of hypobromous acid, and by Willstätter in 1913 as bromine water. Since the concentration of bromine available in these reagents for the bromination of cellulosic material is so small, the number of brominations has to be indefinitely increased. Consequently the process becomes long and laborious. The improved method described in this communication eliminates most of these defects.

The reactions involved are identical in kind with those for chlorine, either gaseous or in solution. Instead of the 'lignone chloride' a corresponding bromide is formed which under acid conditions

possesses a yellow colour like the chloride; it produces a purple colour in cold sodium sulphite, although the intensity of colour is lower than with the chloride, and this colour disappears on heating.

Nascent bromine is liberated according to the following equation:

A similar reaction with chloride and chlorate does not liberate chlorine in the cold but some is evolved on heating. Since 0.75 g. chlorine is used by Norman & Jenkins (1933) at each stage under alkaline conditions, amounts of bromide and bromate can be calculated from the above equation to generate equivalent amounts of bromine in presence of H_2SO_4 . Nearly 1 g. KBr and 0.25 g. KBrO_3 are needed for each stage in the new method. For bromination under alkaline conditions an equivalent amount of NaOH can be calculated from the following equation and added to the acidified mixture:

The use of a bromide-bromate mixture as a source of bromine eliminates the handling of any elementary halogen, and the solution is stable at all temperatures and is easy to prepare. The mixture also supplies bromine easily under either acid or alkaline conditions. For acid brominations bromide-bromate solution is added, followed by a molecular equivalent of H_2SO_4 . For alkaline bromination the equivalent amount of NaOH is added some time after the addition of acid; this delay is necessary since, as judged by the colour and smell of the liberated bromine, it takes some time for bromine liberation to develop.

EXPERIMENTAL

Solutions required: (1) *Bromide-bromate solution.* To a 10% solution of KBr add 2.5 g. of KBrO_3 /100 ml. of solution; warm gently to dissolve the bromate. (2) 10% w/v H_2SO_4 . (3) 5% NaOH. (4) 6% Na_2SO_3 .

Comparison of various procedures in cellulose estimation

1 g. of pulverized cane-reed passing through a 64-mesh sieve was treated according to the recommendations of Norman & Jenkins (1933), substituting NaOBr or a mixture of bromide-bromate for NaOCl. The mixture was allowed to stand for 15 min. instead of the 10 recommended by these authors, washed with water on a poplin filter and then transferred to a beaker, the volume being made up to 100 ml. with 3% Na_2SO_3 . Several experiments were

conducted with different permutations and combinations of acid and alkaline brominations to obtain the cellulose content which was the nearest approach to the value obtained by the Norman & Jenkins hypochlorite method, bearing in mind the effect of such treatments on the cellulosan and apparent lignin content of the cellulose product. The results of some of these experiments are given in Table 1.

Table 1. *Comparison of results with various combinations of brominations under acid and alkaline conditions, with cane-reed (Pennisetum sp.)*

Results expressed in g./100 g. of original material.
Acid and alkaline treatments are indicated by A and N respectively.

Treatment	Cellulose product	Xylan in cellulose product	Lignin in cellulose product	True cellulose
(1) NaOCl in 5N-alkali	42.52	11.25	1.16	30.11
(2) NaOBr in 5N-alkali	42.60	11.22	1.50	29.92
(3) NaOBr in 8N-alkali	30.94	8.84	1.56	29.54
(4) 6A-1N-1A*	42.20	10.08	1.20	30.90
(5) 2N-2A- $\frac{1}{2}$ A- $\frac{1}{2}$ N†	44.54	9.98	2.00	32.56
(6) 3N-2A-1N	47.46	12.30	2.24	32.92
(7) N-A-N-A-N-A-N	43.00	10.04	1.40	31.56
(8) 1N-5A	51.86	14.70	1.80	35.36
(9) 2A-2N-A-N	44.70	11.05	2.32	31.33

* I.e. six brominations in acid, followed by one in alkali and then one in acid.

† $\frac{1}{2}$ A- $\frac{1}{2}$ N indicate that each treatment lasted for 7-8 min. with no intermediate sulphite boil.

It will be noted from Table 1 that NaOBr of the same alkaline strength as the NaOCl gave similar results in respect of every constituent. It should, however, be mentioned that, with the use of NaOBr, one more acid treatment was needed than with NaOCl, in order to obtain a product which gave negligible colour in cold sulphite. NaOBr of a higher alkaline concentration affected the cellulosan fraction adversely. With a bromide-bromate mixture, six acid treatments followed by one alkaline and one acid bromination gave results comparable with those obtained by the hypochlorite method. The appearance of the product after alkaline treatment is always lighter in colour than after an acid treatment. The alkaline product does not give a purple colour in cold sulphite.

Treatment (4) gave a result approximating most nearly to that from the NaOCl method. Bromination under these conditions was found to be harmless to pure cellulose. This was tested by brominating finely ground Whatman no. 1 filter paper. Controls with equal numbers of sulphite and

water extractions were conducted simultaneously. It will be noted from Table 2 that the amounts of cellulose recovered after bromination and sulphite extractions were similar to those obtained from sulphite or water extractions.

Standard method

Based on the above experiments, the following method has been evolved and made applicable to different kinds of materials. The material should be in a uniform state of division; 64-80 mesh seems quite suitable, but for tender green materials 64 mesh is preferable, since the cellulose product tends to gelatinize as bromination proceeds and the filtration is then extremely slow. 1-2 g. of the material, depending upon its age and nature after alcohol-benzene extraction, are stirred in 100 ml. of 3% Na_2SO_3 , brought to the boil and filtered. The washed material is then transferred back to the beaker with 100 ml. of water, and 10 ml. of bromide-bromate mixture are added, followed by 5 ml. of 10% (w/v) H_2SO_4 . The mixture is allowed to stand 15 min., with occasional stirring. The liquor is then sucked off on a cloth filter and the material washed with water. The washed material is retransferred to the container with 50 ml. of water and 50 ml. of 6% Na_2SO_3 are then added. The contents are brought to the boil and the beaker left in a boiling water-bath for 20 min. The process of acid-bromination is repeated till the purple colour due to 'lignone bromide' in cold sulphite is very faint. This may take five to six acid-brominations, depending upon the age and nature of the material. The cellulose product, which is by this time much lighter in appearance, is then suspended in 100 ml. of water, to which are added 10 ml. of bromide-bromate mixture followed by 10 ml. of H_2SO_4 . After the development of the reaction, 5% NaOH (about 10 ml.) is added till the liquor in the beaker is colourless; 15 min. are allowed for alkaline bromination. The material, after being washed, presents a bleached appearance and it is extracted in 100 ml. of 3% Na_2SO_3 as before. In the absence of a simple and reliable test for the removal of lignin, one more acid treatment is given to ensure that the material is completely delignified, as indicated by the absence of purple coloration in cold sulphite extract of the brominated material. Sodium sulphite is finally removed by boiling the final cellulose product in about 250 ml. of water for 2 min. After thorough washing on the filter the product is transferred by a spatula to a weighed Gooch crucible with a cloth disk. This facilitates rapid filtration in the crucible. The crucible is dried at 100° and weighed, and the product is powdered for furfural and lignin determination.

Though the method is somewhat longer than the hypochlorite method, when the reagent for the latter is not easily available, bromide-bromate solution for halogenation appears to be satisfactory. Moreover, for cellulose determination in tender green materials which are not highly lignified, bromine has an advantage over chlorine in that, owing to its lesser reactivity, there is less chance of the tissue being damaged by over-halogenation.

Table 3 shows results obtained for certain materials by the new method, compared with the corresponding figures obtained by the Norman & Jenkins (1933) procedure. From the evidence it is apparent that the method is capable of giving results comparable with those of the hypochlorite determinations. The method can also be applied to large-scale preparations in the same manner as the hypochlorite process.

Table 2. *Results of estimations on pure cellulose (filter paper)*

Treatment	Cellulose recovered from 1 g. of filter paper
6A-1N-1A*	0.974
N-A-N-A-N-A-N	0.975
Six boilings in Na_2SO_3 (20 min. each)	0.975
Six boilings in water (20 min. each)	0.979

* I.e. six brominations in acid, followed by one in alkali and then one in acid.

Table 3. Comparison of results obtained by the hypochlorite method and our new method

Results expressed in g./100 g. original material

Material	Norman & Jenkins method			
	Cellulose product	Xylan in cellulose product	Lignin in cellulose product	True cellulose
<i>Tephrosia vogelii</i>	25.00	1.28	2.32	21.40
Grass cuttings	34.40	6.62	2.08	25.70
Tea leaf	18.34	2.37	2.38	13.59
Paddy straw	41.76	7.51	3.00	31.25

Material	Bromination method			
	Cellulose product	Xylan in cellulose product	Lignin in cellulose product	True cellulose
<i>Tephrosia vogelii</i>	23.20	0.98	1.80	20.42
Grass cuttings	33.00	6.00	2.40	24.60
Tea leaf	19.14	2.23	3.02	13.89
Paddy straw	40.66	6.95	2.72	30.99

SUMMARY

1. A new method for the determination of cellulose in plant materials, based on the use of bromine

as a halogenating agent for lignin, is described. Bromine is liberated in the nascent state in contact with the material by acidifying a mixed solution of bromide and bromate. Bromination under alkaline conditions can also be effected by adding an equivalent amount of NaOH to the acidified mixture. The reactions involved in the process are similar to those in the hypochlorite methods. Results obtained by the use of either NaOBr or a mixture of bromide and bromate are comparable to those given by the NaOCl method.

2. The method is distinctly advantageous in the tropics, where it is difficult to obtain and store NaOCl. One or two treatments (depending upon the nature and age of material) in excess of those needed in the NaOCl method are required, since bromine is less reactive than chlorine. This lesser reactivity is an advantage in the case of tender green plant tissues, which are liable to damage by over-chlorination.

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Palm Oil Carotenoids

3. EXAMINATION OF THE LIPOID PIGMENTS PRESENT IN 'MALAY' AND 'BISSAO' PALM OILS

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The new type of palm grown on plantations in the Malay Peninsula was especially selected to furnish an oil of much paler tint than the palm oil produced commercially in West Africa. It was therefore of interest to compare the carotenoids present in Malay oil with those which occur in a deep red palm oil such as that obtained from the Portuguese island of Bissao.

Samples of both types of oil were examined on the lines described in our first paper (Hunter & Scott, 1941). Chromatographic analysis of the petrol-soluble unsaponifiable matter of 'Malay' palm oil furnished crystalline specimens of α - and

β -carotene. Evidence was also obtained of the presence of lycopene, γ -carotene, and the carotenoid which is adsorbed at a position intermediate between γ - and β -carotene on the chromatogram (Hunter & Scott, 1941). The ether-soluble unsaponifiable matter furnished somewhat ill-defined xanthophylls having absorption spectra similar to those of the neoluteins (Zechmeister & Tuzson, 1938).

The amount of 'Bissao' palm oil at our disposal was very limited, but an examination of this indicated the presence of lycopene, neolycopene and γ -carotene in addition to the α - and β -carotenes which are mainly responsible for the deep red colour.