CCCI. STUDIES ON HEMICELLULOSES.

V. ADDITIONAL NOTES ON THE HEMICELLULOSES OF MAIZE COBS.

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THE method of fractionation of the hemicelluloses of cell wall substances proposed by Norris & Preece [1930] would appear to have been adopted with few major modifications by other workers in this field. Briefly, the sodium hydroxide extract of the cell wall material is precipitated in three stages: by addition of acetic acid; half a volume of acetone; and one volume of acetone. Each of these fractions may again be subdivided by treatment with Fehling's solution, those fractions recovered from the insoluble gel being designated A_1 , B_1 , C_1 , whilst those obtained from the copper solution are referred to as A_2 , B_2 , C_2 .

In order to avoid confusion in questions of classification raised, for example, by Buston [1935], it seems desirable to emphasize the fact that the systematic designations indicate not definite classes of hemicellulose but particular methods of preparation. It has never been suggested that any one fraction represents a particular hemicellulose of definite chemical entity or that hemicelluloses of the same designation but prepared from different plant sources are necessarily chemically identical. The fractionation is very largely physical and it is more than probable that the several fractions usually obtained still comprise more or less complex mixtures.

Evidence obtained during the course of experiments on the hemicelluloses of the flowers of the hop, described in a subsequent paper of this series, would appear to indicate that in the past the precipitation at some stages has been incomplete, and especially is this the case where the first fraction, precipitable by acetic acid, is concerned. Thus, in precipitating fraction A from the NaOH extract, the original directions proposed addition of glacial acetic acid until the mixture was acid, but the actual amount necessary was not specified. It has been found that the pH of precipitation has a considerable effect on the amount of hemicellulose precipitated, and it will be appreciated that a considerable volume of acetic acid is necessary in order to lower the pH of the mixture to any extent, since a buffered sodium acetate-acetic acid mixture is produced. It seems very probable that in the preparation of maize cob hemicelluloses [Preece, 1930] a considerable proportion of fraction A escaped precipitation at the acid stage and appeared later as fractions B and C.

Maize cobs were chosen for the present investigation since they were most readily available, but there is reason to believe that the pH of the solution at precipitation is of general importance. Thus the precipitability of hemicelluloses from hops was found to depend on the pH of the medium and, moreover, the optimum pH in this case differed from that in the case of the maize cob products. It is, in fact, conceivable that the pH of minimum solubility of the hemicelluloses is as definite a physical characteristic as the isoelectric point of proteins.

The precipitation of the fractions by copper solutions was also examined in the case of maize cob hemicelluloses. The solutions used were Fehling's solution,

Schweitzer's solution and a mixture of glycerol and cupric sulphate. For the decomposition of the gels both dilute hydrochloric and acetic acids were used. It was found that the mixture of glycerol and cupric sulphate was the most effective precipitant, whilst for the decomposition of the gel, acetic was to be preferred to hydrochloric acid.

EXPERIMENTAL.

Precipitation of hemicellulose A₁: effect of pH on yield.

In a preliminary experiment the hemicellulose A₁ prepared by Preece [1930] was used in 1 % solution in 4 % NaOH. 5 ml. portions of this solution in a series of test-tubes were made up to 20 ml. with distilled water and acetic acid in amounts sufficient to bring the solutions to a range of pH values varying from 7 to 3 at intervals of 0.5 (determined colorimetrically). Turbidity was observed in each tube almost immediately, but on standing overnight the precipitate settled out completely in only one tube, namely that whose contents were adjusted to pH 4. On further standing, precipitates in the other tubes settled out gradually, but the most complete precipitation was still obtained at pH 4.

The results of the preliminary qualitative study were confirmed by quantitative experiments on a larger scale. In this case, the maize cobs were treated in the usual manner for the preparation of a hemicellulose extract in 4 % NaOH. 500 ml. portions of the extract were used and made up with acetic acid and distilled water to 800 ml. so as to give a series as before, each mixture at a different pH. The pH was determined electrometrically, using the quinhydrone electrode. The mixtures were allowed to stand for 7 days, after which the precipitates were filtered off, washed free from acid by 50 % alcohol, dried as usual and weighed. Each sample contained about 1 % lignin and ash varying between 1.4 and 2.9 %. The yields obtained, on a lignin- and ash-free basis, were as follows:

pH at precipitation	Yield (g.)
6.0	11.6
5.4	11.7
4.7	12.4
4.1	14.2
3.7	14.6

The increased amount precipitated at pH 3.7 as compared with that at 4.1 is counterbalanced by a considerably increased difficulty in filtration. On the large scale it was found to be exceedingly difficult to separate the precipitate obtained at pH 3.7 even at the centrifuge, whereas that obtained at 4.1 presented no such difficulty. It was therefore concluded that the optimum pH for the precipitation of the hemicellulose was 4.0–4.1.

Composition of the precipitates.

It was found that there was no significant difference in the yields of furfuraldehyde from the precipitates obtained at different pH values, and hence the increased yields observed represent definite improvements in yield of the same substance or mixture of substances. A typical sample dried to constant weight at 98° contained 1.72 % ash and 1.26 % lignin. Uronic anhydride and furfuraldehyde were determined, and the percentage of xylan, *X*, was calculated from the formula $X = 0.0060 + 0.7859P - 1.146C$, where *P* = phloroglucide and *C* = carbon dioxide from uronic anhydride. [Norris & Resch, 1935; Angell *et al.*, 1936.]

In this way the composition was found to be:

	%
Xylan	94.8
Glycuronic anhydride	5.1
Methoxyl	0.5

Now the yield of this fraction A_1 obtained by precipitation at pH 4 corresponded to 14.5% of the dry maize cobs. For comparison we may quote the results obtained by Preece [1930]:

Fraction	Yield %	Methyl- pentosan %	Furfural- dehyde %	Uronic anhydride %
A_1	8.0	Nil	61.49	3.76
B_1	6.0	7.01	57.31	5.20
C_1	1.0	7.42	54.57	7.40
C_2	0.5	8.91	43.90	4.56

It will be seen that our yield of fraction A_1 accounts for Preece's fractions A_1 , B_1 and possibly C_1 . Each of these latter fractions was closely similar in composition, consisting chiefly of xylan and glycuronic anhydride. We have been unable to confirm the presence of methylpentosan suggested by Preece. This suggestion was based largely on the fact that about 7% of the phloroglucide was soluble in hot alcohol, giving a brownish solution. We also have found that the alcoholic solution varied in colour from green to brown, but in no case was the absorption band characteristic of methylfurfuraldehydephloroglucide detected. Furthermore, Rosenthaler's [1909] reaction gave negative results on the hemicellulose and its hydrolysis products.

Thus, Preece's fractions A_1 , B_1 and C_1 are in reality one fraction only, A_1 ; fraction C_2 appears to have a separate existence since in this case the xylan is replaced by araban.

Precipitation by copper solutions.

The sample of hemicellulose A_1 used in these experiments contained 2.85% of ash and 0.94% of lignin. A solution was prepared containing 1% (on an ash-free basis) of the hemicellulose in 4% NaOH. 200 ml. portions of the solution were measured out, precipitated with a copper solution and decomposed with acid, the following reagents being used:

For precipitation: (a) 10 ml. glycerol followed by 20 ml. *M* cupric sulphate; (b) 60 ml. of Fehling's solution; (c) 30 ml. of Schweitzer's reagent.

For decomposition: (d) 200 ml. 25% acetic acid; (e) 200 ml. *N* HCl.

In each case, after addition with stirring of the precipitating reagent, the solution was allowed to stand overnight.

The copper gel was then filtered off on a hardened paper and subsequently decomposed with 200 ml. of acid, the solution being stirred mechanically. Half a volume of acetone was added to the acid solution and after standing overnight the precipitate was filtered off on a Berlin funnel, washed with 50% acetone until free from acid and copper and dried to constant weight as usual. The following yields (ash-free) were obtained:

Precipitation by	Decom- position by	Yield		Ash %
		g.	%	
Glycerol and cupric sulphate	HOAc	1.75	87.2	1.20
" "	HCl	1.54	76.8	0.60
Fehling's solution	HOAc	1.43	71.0	0.73
" "	HCl	1.39	69.0	1.00
Schweitzer's reagent	HOAc	1.42	70.8	2.64

As in the case of the acid precipitations, the chemical composition of all the products was identical within the usual experimental error, so that the larger yields in the case of glycerol and cupric sulphate represent genuine improvements in technique.

Buston [1934], working with a hemicellulose mixture from cocksfoot grass, commented on considerable losses of hemicellulose when precipitating fraction A and when carrying out the copper precipitation. It seems quite probable that losses in the former case were due to lack of regulation of the pH at which precipitation took place, and the above results demonstrate sufficiently conclusively that losses at the copper precipitation stage may be minimized by the use of glycerol and cupric sulphate, followed by decomposition with acetic acid. In this way the amount of inorganic salt introduced, and hence the loss on subsequent washing, is minimized. It should be pointed out that although the hemicelluloses of maize cobs investigated by Preece have been the subject of special comment in this paper, similar remarks probably apply to other hemicelluloses which have not yet been re-examined.

SUMMARY.

1. There is an optimum pH for precipitation of fraction A of a hemicellulose mixture.
2. Failure to precipitate at the optimum pH leads to a low yield of fraction A and the appearance of this fraction at later stages in the separation.
3. Improved yields of hemicellulose fractions may be obtained by precipitating with glycerol and cupric sulphate instead of Fehling's solution, and by subsequent decomposition with acetic acid instead of hydrochloric acid.

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