36. INFLUENCE OF ALKALINE TREATMENTS AND PRETREATMENTS IN HEMICELLULOSE PREPARATION

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THE method for the preparation of hemicelluloses originally proposed by Norris & Preece [1930] involves a preliminary treatment of the tissue material with 0.5% ammonium oxalate solution for the removal of pectin and water-soluble substances followed by treatment with boiling 50% ethyl alcohol-1% NaOH solution with the intention of diminishing contamination of the subsequent preparation with lignin. Hemicelluloses are then removed from the residue by solution in 4% aqueous NaOH.

It was pointed out later by Preece [1931] that the alcoholic-NaOH treatment may remove from the tissue a notable amount of furfuraldehyde-yielding material. The material so removed was tentatively designated "lignosaccharide"; that from box-wood showed marked resistance to hydrolysis, but otherwise there was found no reason to distinguish it from other structural hemicelluloses which, themselves, show varying degrees of resistance to the action of boiling dilute acid.

Alcoholic-NaOH pretreatment in the preparation and determination of hemicelluloses has been criticized by Norman [1935; 1937, 1, 2]. Thus, it is claimed that the amount of material so extracted varies according to the concentrations of alcohol and alkali used. The objection is a sound one. Again, use of the term "lignosaccharide" is objected to on the ground that insufficient difference of properties is shown between this material and other hemicelluloses to justify the introduction of a further complication into the already confused nomenclature of these substances. This objection is perhaps less sound for, if it is borne in mind—as has been expressly emphasized [Preece, 1931]—that the term implies a structural hemicellulose prepared by the method described, there can be little objection to its use until the relationship between this and other hemicellulose fractions is more clearly understood.

In an investigation of the hemicelluloses of the hop flower [Angell & Norris, 1936] the alcoholic-NaOH pretreatment was retained, the authors stating that furfuraldehyde-yielding substances could not be detected in the extract. Norman [1937, 1], however, states that the treatment involves a loss of such materials, even though furfuraldehyde itself cannot be obtained from the extract. Figures supporting this contention are presented, and the statement is made that the only true criterion of preservation of furfuraldehyde is a proof that the tissue residue shows no reduction in furfuraldehyde yield. This suggests interesting possibilities, with alkali treatment apparently resulting in a destruction of furfuraldehyde-yielding substances. The following hitherto unpublished figures for a 9-10-year-old sample of lilac wood certainly appear to support the idea of such destruction. The analysis was carried out by the method

of Preece [1931], and the tissue residue after removal of "combined hemicellulose" represented approximately 57.5% of the original dry wood; it gave 6.22% of its own dry weight of furfuraldehyde, corresponding to a yield on the original dry wood of 3.58% of furfuraldehyde. The figures of Table 1 are

Table 1

Furfuraldehyde from substances soluble in:

(i) 0.5% Ammonium oxalate	%
(a) Pectin	0.37
(b) Non-pectin	0.46
(ii) Alcoholic-NaOH	0.72
Furfuraldehyde from:	
(iii) Free hemicellulose	4.37
(iv) Combined hemicellulose	1.16
(v) Tissue residue	3.58
Total	10.66

calculated on the original dry wood; however, the furfuraldehyde yield found directly was 12.44%. The discrepancy between the two totals is surprisingly high, and it cannot be readily accounted for unless degradative changes have occurred during extraction. It is impossible that as much as 14% of the total furfuraldehyde could be represented by normal "extraction losses".

Turning now to the extraction of other hemicellulose fractions, it may be asked whether it is allowable to follow the extraction with NaOH in the cold with a boiling extraction with this solvent. The original justification of such a proceeding lies in figures such as those for box-wood shown in Table 2, these

Table 2

Furfuraldehyde from:	On dry wood	
	Lilac wood %	Box-wood %
(i) Ammonium oxalate extract	0.83	0.26
(ii) Lignosaccharide and free hemicellulose	5.09	4.34
(iii) Xylan associated with crude cellulose	7.22	5.11
Total	13.14	9.71
Total furfuraldehyde found directly	12.44	11.86

being re-calculated from the results of the analysis already published [Preece, 1931]. It is apparent from the box-wood figures that 2.15% of furfuraldehyde remains unaccounted for; the aim of the boiling NaOH treatment was to endeavour to trace the reason for this though, to be sure, similar summations for other woods (see, for example, the figures included for lilac) exceed the total found directly, which gives obvious evidence of overlapping. Part at least of the box-wood deficit may, in view of what has already been said, be due to the destructive effect of the alkali. However, apparent justification for the treatment is found in the composition of the materials isolated from box-wood as "combined hemicellulose"; thus, the products were of polyuronide hemicellulose type, similar to those extracted by cold 4% NaOH.

It is clear that further study is required of the effects on yield and composition of extractives at varying temperatures and of various concentrations, and of the suggested possibility of a degradative effect of the treatments. An examination of some aspects of the problem is described below.

EXPERIMENTAL

Furfuraldehyde determination

The influence of the various treatments employed is first measured by noting the changes induced in the furfuraldehyde yield. The difficulties inherent in the furfuraldehyde determination are well known, and a valuable résumé of its problems is provided by Dorée [1933]. Again, Angell *et al.* [1936] point out certain difficulties of interpretation of results, and state that it is inadmissible to use the Kröber conversion factors when alcoholic extraction of the phloroglucide precipitate is employed. Circumstances having precluded the use of the distillation method described by these authors, the normal distillation procedure was followed in the present work, but alcoholic extraction of the phloroglucide precipitate was omitted. Furfuraldehyde (f) was calculated from the weight of phloroglucide (p) using the formula: $f = 0.5185 (p + 0.0052)$. It may be noted that the weights of phloroglucide here involved are uniformly of the order 0.06–0.11 g.

Whilst the results presented are possibly somewhat few in number for statistical analysis, it is very desirable when small changes are under examination to give some idea of the errors involved in the determinations concerned. Accordingly, the average variances of the results are given for each series [see Fisher, 1938, p. 53], and from these have been calculated the standard deviations of the means in each case and also the standard deviations of the differences of means. A useful measure of the significance of the results is thus obtained, though it is realized that the standard deviations themselves are probably of limited accuracy. However, application of the “*t*-test” [Fisher, 1938, p. 128] to the differences allows of conclusions as to significance which are in agreement with those obtained by the method shown.

Treatment of sawdust

The material available for examination was a sample of teak sawdust and, in the first series of treatments, this was used in its raw condition. The treatments applied are shown below, each treatment and the subsequent furfuraldehyde determinations being carried out in quadruplicate.

a_1 . The wood (0.5–1.0 g.) was treated with 50 ml. *N* NaOH, and the mixture kept at room temperature for 18 hr. 50 ml. *N* HCl were then added, and furfuraldehyde distillation was carried out after the further necessary adjustment of HCl concentration.

a_2 . A similar mixture of wood and 50 ml. *N* NaOH was gently boiled under reflux for 1 hr. 50 ml. *N* HCl were added, and furfuraldehyde distillation was then proceeded with.

a_3 . The wood was boiled under reflux for 1 hr. with 100 ml. of 50 % alcohol containing 25 ml. of *N* NaOH (i.e. 50 % alcohol–1 % NaOH). The alkali was then neutralized and the alcohol removed by distillation *in vacuo* at the lowest possible temperature; a further 25 ml. each of *N* NaOH and HCl were added before furfuraldehyde distillation.

a_4 . This and the two following treatments represent additional controls for a_3 . After refluxing for 1 hr. with 100 ml. of 50 % alcohol, the alcohol was removed as in a_3 . 50 ml. each of *N* alkali and acid were then added and furfuraldehyde was determined after the necessary adjustments of volume and HCl concentration.

a_5 . As a_4 , but the reflux treatment was with water instead of with 50 % alcohol.

a_6 . As a_5 , again with distilled water; the reflux boiling was omitted, but the vacuum distillation necessary for volume adjustment was retained.

a_7 . It will be noted that in all the above, and in b below, furfuraldehyde distillation was carried out in presence of 2.93 g. of NaCl. To indicate the influence on the furfuraldehyde yield of distillation in presence of this amount of salt, normal determinations were carried out without addition of N NaOH and N HCl.

b . In this general control, the wood was mixed with 50 ml. each of N NaOH and N HCl, and the furfuraldehyde distillation was then immediately proceeded with.

The results from these treatments are shown in Table 3, the furfuraldehyde means being calculated on the dry wood.

Table 3

Treat- ment	Furfuraldehyde (mean) %	$b \sim a$	$b \sim a$ 0.085	Furfuraldehyde yield is
b	8.09 ± 0.060	—	—	—
a_1	8.09 ± 0.060	0.00 ± 0.085	0.0	—
a_2	7.79 ± 0.060	0.30 ± 0.085	3.5	Decreased
a_3	7.82 ± 0.060	0.27 ± 0.085	3.2	Decreased
a_4	8.00 ± 0.060	0.09 ± 0.085	1.1	—
a_5	8.13 ± 0.060	0.04 ± 0.085	0.5	—
a_6	8.09 ± 0.060	0.00 ± 0.085	0.0	—
a_7	8.31 ± 0.060	0.22 ± 0.085	2.6	Increased
Average variance of results				0.014533
Average variance of mean of quadruplicates				0.003633
Standard deviation of mean of quadruplicates				± 0.060
Standard deviation of difference of means				± 0.085

It will be observed that treatments a_2 and a_3 cause significant reduction in furfuraldehyde yield; the vacuum distillation (as in a_5 and a_6) used for removal of alcohol appears to be without influence on the results of a_3 . The influence of a_4 is not of itself significant, though there is a possible contribution here to the loss in a_3 . Treatment a_1 has no measurable effect, nor has a_5 which involves boiling with water. The importance of carrying out the whole of the treatments a_1 – a_6 and b in presence of a standard amount of NaCl is clear, since examination of the results of b and a_7 shows that there is a significant decrease in furfuraldehyde yield in presence of NaCl.

Removal of fatty material is usually recommended as a preliminary to wood analysis, and accordingly a further sample of the sawdust was exhaustively extracted successively with ether and alcohol (95 %), losing thereby 8.64 % of its dry substance. The extracted residue was then submitted to treatments a_2 , a_3 , a_7 and b . The results, shown in Table 4, are calculated to the original, unextracted dry wood.

Table 4

Treat- ment	Furfuraldehyde (mean) %	$b \sim a$	$b \sim a$ 0.096	Furfuraldehyde yield is
b	7.78 ± 0.068	—	—	—
a_2	7.09 ± 0.068	0.69 ± 0.096	7.2	Decreased
a_3	7.45 ± 0.068	0.33 ± 0.096	3.4	Decreased
a_7	7.80 ± 0.068	0.02 ± 0.096	0.2	—
Average variance of results				0.018558
Average variance of mean of quadruplicates				0.004639
Standard deviation of mean of quadruplicates				± 0.068
Standard deviation of difference of means				± 0.096

Treatment a_2 has again resulted in a significant decrease in the yield of furfuraldehyde, the loss in this series being apparently much greater than in the previous one. a_3 , also, has again caused a decrease, but its magnitude is comparable with that of the previous series. Of great interest is the fact that the apparent depressing effect of NaCl on furfuraldehyde yield has now disappeared.

Treatment of hemicellulose

In view of the treatment losses revealed above, it is of obvious interest to know whether similar losses would be encountered with purified hemicellulose. Accordingly, a preparation of free teak hemicellulose was made by the following method. Teak sawdust was extracted with 0.5% ammonium oxalate solution in the usual way, and the residue was extracted at room temperature with successive quantities of 4% NaOH solution, each extraction occupying 2 hr. The extracts were combined, filtered through pulp and acidified with acetic acid. No attempt at fractionation was made, the hemicelluloses being precipitated together by addition of an equal volume of acetone. The washed precipitate was redissolved, and submitted to the purification treatment described below as treatment (iii). Treatments a_2 , a_3 , a_7 and b were carried out on the product, this time in duplicate. The results, calculated on the dry, ash-free hemicellulose, are shown in Table 5.

Table 5

Treat- ment	Furfuraldehyde (mean) %	$b \sim a$	$b \sim a$ 0.72	Furfuraldehyde yield is
b	54.23 ± 0.51	—	—	—
a_2	47.84 ± 0.51	6.39 ± 0.72	8.9	Decreased
a_3	51.25 ± 0.51	2.98 ± 0.72	4.1	Decreased
a_7	53.13 ± 0.51	1.10 ± 0.72	1.5	Decreased ?
Average variance of results				0.5176
Average variance of mean of duplicates				0.2588
Standard deviation of mean of duplicates				± 0.51
Standard deviation of difference of means				± 0.72

It is with some reserve that statistical analysis is applied to these results of duplicate determinations, and the influences of a_2 and a_3 are in fact so clear that such analysis seems unnecessary. However, if the results of Table 5 and the furfuraldehyde figures of Tables 6 and 7 (calculated on dry, ash-free, lignin-free hemicellulose) are combined, 9 duplicate determinations become available, the means of which, with the exception of a_2 of Table 5, occupy the very limited range of 51.25–55.40. It seems reasonable, therefore, to calculate an average variance of the mean of duplicates from these figures, and hence to determine the appropriate standard deviations. It is felt that considerable confidence can be placed in the figures so obtained; their magnitudes are shown with Table 5. It will be seen that the differences $b-a_2$ and $b-a_3$ are clearly significant, whilst that of $b-a_7$ is of doubtful significance.

Hemicellulose purification methods

Since the object of the alcoholic-NaOH treatment as a preliminary to hemicellulose extraction was to reduce the lignin content (or the possibility of lignin contamination) of the subsequent hemicellulose preparations, it was desired to obtain information as to the extent of such contamination when the treatment was omitted. Further, in view of the solubility of lignin in solvents such as

alcohol, the possibility exists that such solvents used in the precipitation and drying of hemicelluloses might themselves reduce the contamination to a low figure [cf. Norman, 1937, 1]. With a view to using conditions which would favour the dissolution of lignin, sawdust was extracted for 1 hr. with boiling 4 % NaOH. The extract after filtration through pulp was acidified with acetic acid. No fractionation was attempted, and an equal volume of acetone was immediately added. The acetone, whilst precipitating hemicellulose, had the effect of redissolving the bulk of the brownish precipitate—presumably lignin—thrown down by the acid. The precipitated hemicellulose, after washing with 50 % acetone, was taken to dryness by alcohol treatment and divided into three portions:

(i) Control: crude hemicellulose preparation.

(ii) This portion was redissolved in 4 % NaOH, the solution neutralized with acetic acid and the hemicellulose reprecipitated with an equal volume of acetone. The product was washed with 50 % acetone and thereafter taken to dryness.

(iii) After redissolving in 4 % NaOH solution, an equal volume of mixed Fehling's solution was added and a volume of acetone also equal to that of the original solution. The precipitate was dissolved in 2*N* HCl and the hemicellulose reprecipitated with 1.5 vol. acetone. The precipitate was washed with (a) dilute HCl containing 60 % of acetone and (b) 60 % acetone. These washings are very necessary if a maximum elimination of ash is to be achieved. The product was then taken to dryness with the usual alcohol treatment.

The three products, distinguished by the above numbers, were investigated for ash, lignin content and furfuraldehyde yield. The lignin determination was made by the 72 % H₂SO₄-18 % HCl method of Schwalbe as used earlier [Preece, 1931]. It has been objected that the method probably tends to give results rather in excess of the true values; this, however, is of little importance in the present instance, where the error (if any) is likely to be constant in the comparative series concerned. Furfuraldehyde was determined by the normal distillation process. The results are shown in Table 6. It was also intended to carry out protein determinations on all three products. However, product (i) was found to contain not more than 0.08 % N which, with the quantities available for examination, lay within the limits of experimental error. Accordingly, further protein determinations were not proceeded with.

Table 6

	Preparation no.		
	(i) %	(ii) %	(iii) %
On dry hemicellulose:			
Ash	4.74	4.06	0.22
Lignin	2.53	2.69	2.02
Furfuraldehyde	47.75	49.13	52.42
On dry, ash-free hemicellulose:			
Lignin	2.68	2.80	2.03
Furfuraldehyde	50.11	51.16	52.50
On dry, ash-free, lignin-free hemicellulose:			
Furfuraldehyde	51.49	52.63	53.58

Taking preparation (i) as the standard, the furfuraldehyde value (corrected for ash and lignin) is significantly increased by the copper treatment but not by simple re-solution and reprecipitation. The lignin figures are too few in number for reliable analysis, but it would appear unsafe to assume that any clearly

significant changes in lignin content have occurred as a result of the purification methods employed. The influence of these methods on the ash content of the products is too clear to need comment.

Alcoholic-NaOH pretreatment and hemicellulose composition

Finally, two preparations of free hemicellulose (unfractionated) were made from teak. Preparation (iv) was made by the method of Norris & Preece [1930], but omitting the alcoholic-NaOH pretreatment; preparation (v) was obtained using the full method of these authors. Both products were purified by method (iii) described in the previous section, and the results of analysis of the purified products are shown in Table 7.

Table 7

	Preparation no.	
	(iv) %	(v) %
Yield, on dry wood:		
Dry, ash-free hemicellulose	1.29	1.33
On dry, ash-free hemicellulose:		
Lignin	2.28	0.81
Furfuraldehyde	53.30	54.95
On dry, ash-free, lignin-free hemicellulose:		
Furfuraldehyde	54.75	55.40

Clearly, the alcoholic-NaOH pretreatment has resulted in an important decrease in the lignin content of the preparation concerned. On the present figures, it cannot be assumed that the apparent increase in furfuraldehyde due to the pretreatment is significant. It may be noted that the lignin content of preparation (iv) is of the same order as that of each of (i), (ii) and (iii). Further, the furfuraldehyde content of the dry, ash-free preparation (iv) agrees well with the corresponding figure of a_7 , Table 5. The yields of (iv) and (v) are substantially identical.

DISCUSSION

It is apparent that treatment of plant materials with hot alkaline solutions may result in considerable modification of the furfuraldehyde-yielding substances present, leading to marked decreases in furfuraldehyde yield. A similar effect is seen with hot alkaline treatments of extracted hemicelluloses. In the present experiments with raw wood, boiling 4% NaOH and boiling 50% alcohol-1% NaOH give effects of similar magnitude, but with ether-alcohol-extracted wood and with the isolated hemicellulose mixture the influence of boiling 4% NaOH is approximately double that of the alcoholic NaOH. The actual losses with boiling 4% NaOH are, expressed as percentages of the total furfuraldehyde, 3.7, 8.9 and 11.8% for raw wood, extracted wood and isolated hemicellulose respectively. For the alcoholic NaOH treatment the corresponding figures are 3.3, 4.2 and 5.5%. It is noteworthy that the losses, particularly with the former treatment, increase markedly as the purity of the furfuraldehyde-yielding material increases. Whilst there is no direct evidence to explain this, it may well be that concomitant substances exert a protective action which is probably at least partly mechanical, since pre-extraction of wood with ether-alcohol definitely increases the effect of aqueous NaOH.

For obvious purposes of control, the principal determinations shown in Tables 3, 4 and 5 were carried out in presence of NaCl, and the variable influence of the salt is not without importance. NaCl showed a significant inhibiting effect in the raw wood and no detectable change in the extracted wood, whilst with the

pure hemicellulose it gave an increase in yield, statistically of doubtful significance. Such a result was not anticipated, it having been expected that NaCl would give a regular increase in yield throughout. It is not proposed to discuss in detail the probable reasons for the facts observed; possibly, other concomitants in addition to NaCl are capable of influencing the furfuraldehyde yield, the observed effect depending on the sum of the influences (positive or negative) of each.

A variety of circumstances has doubtless contributed to the fact that the figures for ether-alcohol-extracted wood are regularly somewhat lower than those for raw wood. Factors such as those above might play a part, as might also the solution of furfuraldehyde-yielding substances in these solvents; the latter possibility is of little importance in the case of woods. More important is the possibility of distillation with the furfuraldehyde of fatty materials, which would accordingly tend to produce an artificially high result. With teak the influence of such materials is probably unusually high. It seems desirable that, for high accuracy, this possibility should be borne in mind when carrying out the determination on natural products.

Turning now to the isolated hemicelluloses themselves, a number of significant facts emerge. Contamination with protein is of negligible proportions when mature wood is the raw material used. Small amounts of lignin pass into the preparations, and cannot be eliminated by copper treatment. It is of interest that the material determined as lignin is substantially less in amount when alcoholic-NaOH is used for pretreatment, and there is accordingly justification for the original suggestion that such pretreatment would result in enhanced purity, at least so far as lignin content is concerned. The pretreatment in the present case has neither increased nor decreased the hemicellulose yield, and the furfuraldehyde yield from the hemicellulose though apparently slightly higher is not significantly so. The degrading effect of the alkali might have been expected to cause a decrease in hemicellulose yield, but the loss due to this has been counterbalanced by increased dissolution of other material.

How far should these results be allowed to influence the choice of methods for hemicellulose preparation? Simple solution of hemicellulose material during the pretreatment is not necessarily disadvantageous if it is recognized that such extraction does take place; incidentally, no important extraction of a lignosaccharide was demonstrable in the case of teak. On the whole, it would appear that the advantages of the treatment are outweighed by its disadvantages, and it should therefore be omitted. In its place, extraction with alcohol alone might be useful in some cases for the removal of pigments, fatty materials, and the like. Alternatively, alcoholic-NaOH treatment in the cold as used by Buston [1934] may have advantages with certain materials [cf. Norman, 1937, 2, p. 57], so long as its possible solvent action for hemicelluloses is examined in each case. It must not be assumed that it is no longer necessary to investigate the relationship between lignosaccharide (or, if it be preferred, hemicelluloses soluble in hot alcoholic-NaOH) and those dissolved by cold NaOH solutions.

Clearly, cold 4% aqueous NaOH is unobjectionable for use as a hemicellulose extractive agent when judged by the absence of degradative effect, but the position is otherwise if this solvent is used at raised temperatures. However, the difficulty already cited at the outset of this paper still persists, viz. that cold 4% NaOH does not in all cases remove the whole of the polyuronide hemicelluloses. Until this difficulty is overcome it may be necessary, despite its disadvantages, to retain the treatment when it succeeds in extracting polyuronide material. If, in fact, it has the added disadvantage of also dissolving cellulosan, e.g. of the para-xylan type, this is to be regretted but must be accepted.

SUMMARY

1. Using teak sawdust as raw material, and from the evidence of important reductions in furfuraldehyde yield, it is shown that boiling alcoholic and aqueous NaOH solutions exert degradative effects on hemicelluloses.

2. Pretreatment with boiling alcoholic NaOH may reduce the lignin content of subsequent hemicellulose preparations, but the disadvantages of this pretreatment outweigh its advantages.

3. In view of the limited solvent action of cold 4% NaOH, it may not be possible at present to dispense with boiling extraction in all cases.

4. The influence of concomitant substances on furfuraldehyde yield from hemicelluloses is noted.

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