CXLVII. THE BIOLOGICAL DECOMPOSITION OF PLANT MATERIALS.

I. THE NATURE AND QUANTITY OF THE FURFUR- ALDEHYDE-YIELDING SUBSTANCES IN STRAWS.

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INTRODUCTORY.

THE biological decomposition of cellulosic materials is a process which is obviously bound up with the chemical composition of the plant tissues, and the availability of the constituents to the members of the micro-flora present. Rege [1927] states that two factors appear to control the decomposition of cellulosic material in the presence of sufficient assimilable nitrogen. The first is the food or "energy" factor, which he associates with the "pentosans," and the second, the "inhibitory" factor, which is the lignin fraction. He claims that it is possible to predict the decomposability of any material, for if the ratio of "energy" factor to "inhibitory" factor, that is "pentosans" to lignin, is more than unity, the material is easily decomposed, but if it is below 0.5 the material is very resistant to microbial attack. Work on somewhat similar lines has been carried out by Waksman and Tenney [1926, 1927, 1, 2, 1928]. They recognised that the most resistant constituent of plant tissues is the lignin fraction, which accumulates during the process of rotting, whilst the soluble materials, the cellulose and the pentosans, are progressively removed.

In the investigations to be described in a further communication, the point at issue was the relative importance of the various furfuraldehyde-yielding substances and cellulose. For this purpose a method of differentiating these substances had to be devised. Both Rege [1927] and Waksman and Tenney [1926] obtained a figure for "pentosan" by employing the Kröber factor on the total furfuraldehyde yield. Figures obtained in this way have little or no significance, since they bear no relationship to the actual quantity of furfuraldehyde-yielding constituents of the particular material under investigation. In point of fact, low results are invariably obtained, since the use of this factor involves the assumption that the furfuraldehyde-yielding substances present consist solely of pentose units, an assumption which cannot possibly be defended in view of the large amount of experimental evidence to the contrary. Furfuraldehyde may be obtained from two groups on

distillation with mineral acids—the pentose unit yielding about 75 % of the theoretical amount, and the uronic acids, giving 33 % of their weight. These groups both occur together with hexose units in various proportions in the hemicelluloses, gums, and pectin. The presence of hexose units obviously lowers the furfuraldehyde yield of the product below that which the Kröber factor would indicate. For example, 20 parts of furfuraldehyde might be derived from about 32 parts of xylan, from 100 parts of pectin, or from 61 parts of the hemicellulose A from rye straw, and the use of the Kröber factor, which in this case would indicate 35 % of “pentosan” is manifestly meaningless. In the work the source of the furfuraldehyde has in each case been investigated, so that the figures for the furfuraldehyde-yielding constituents may be obtained as a close approximation to the quantity actually present.

METHODS OF ANALYSIS.

The material for analysis was evenly but not too finely ground, and the following analyses carried out.

Total furfuraldehyde yield. The standard method of boiling with 12 % hydrochloric acid was followed, the furfuraldehyde being precipitated with phloroglucinol. The phloroglucide was extracted with boiling alcohol to remove traces of the phloroglucides of methylfurfuraldehyde and ω -hydroxymethylfurfuraldehyde.

Carbon dioxide yield. The modification of Lefevre and Tollens's [1907] method as proposed by Nanji, Paton and Ling [1925] for the estimation of uronic acids was employed.

Calcium pectate yield. Since it is not possible to determine the actual form in which pectin is present in the straw it is conveniently expressed as calcium pectate, being estimated by extraction with 0.5 % ammonium oxalate according to the method of Nanji and Norman [1928].

Cellulose. Despite certain objections which will be discussed later, the chlorination method due to Cross and Bevan [1918] was employed with certain modifications. The chlorination was actually carried out on a small Büchner funnel, so that a stream of the gas could be drawn through the straw and proper penetration effected. The preliminary treatment for 20 minutes with 100 cc. of 1 % NaOH as originally proposed by Cross and Bevan was employed, even though this has been adversely criticised by several workers, notably Renker [1910], on the grounds that the cellulose yield is lowered thereby. It does, however, serve to remove a portion of the encrusting hemicelluloses, so that the chlorine may have full access to the lignin, and in this way, and also, because it ensures complete wetting, lowers the time necessary for chlorination. Over-chlorination is to be avoided owing to oxidation, and the long periods used by some workers are unnecessary if care is taken to ensure proper penetration. For the straw used in this work, three periods of 12, 7 and 5 minutes were found to be sufficient to free the cellulose from

lignin. The final washing must be very thoroughly carried out with hot water, since, if washing is unsatisfactory, charring takes place on drying, owing to the traces of sulphuric acid formed from the permanganate and sulphurous acid, after the bleaching process.

The product obtained by the chlorination method cannot be regarded as consisting solely of pure cellulose. The principle of the estimation is the resistance of certain of the cell wall substances to attack by chlorine and sulphite, and in this the mechanical structure of the tissue is not unimportant. It is found that the "cellulose" isolated by the Cross and Bevan method from plant tissues almost invariably yields a considerable quantity of furfuraldehyde, due to furfuraldehyde-yielding substances intimately associated with the cellulose. Many workers correct the percentage yield of cellulose for "pentosan" using the Kröber factor, thus obtaining a somewhat truer estimate of the real cellulose content. This adjustment has been criticised on the grounds that the furfuraldehyde may be in part derived not from pentosans but from oxidation products formed from cellulose during the chlorination. This objection will be discussed later.

Lignin. The usual methods for the estimation of this constituent depend on the fact that it is chemically very resistant, and that by treatment with strong acid all other substances are destroyed, leaving the lignin alone. It is certain however that the lignin is changed in some way by this treatment since the final product differs in methyl and acetyl content and is insoluble in sulphite. A more direct method, by extraction with alkali under pressure, has been proposed by Mehta [1925], but this, though theoretically sound, appears to remove only a certain portion of the lignin, the results being considerably lower than by the acid methods. Willstätter and Zeichmeister [1913] proposed the use of hydrochloric acid of *d.* 1.21, but this has the disadvantage that, unless ice is used for cooling, the strength of the acid diminishes and the results are high. The method due to Ost and Wilkening [1910], involving the use of 72 % sulphuric acid, gives results which are more consistent; and a modification of it put forward by Schwalbe [1925] was employed in this work. 2 g. of the dried material are treated with 60 cc. of 72 % H_2SO_4 and 15 cc. of 18 % HCl for 24 hours, the whole being kept in a cold water-bath. At the end of this period, about 500 cc. of distilled water are added and the whole is boiled for half an hour. This final treatment completes the hydrolysis leaving a product which filters readily on a Gooch crucible through paper. After thorough washing it is dried, weighed and ashed, the weight of lignin being corrected for the ash present, which is usually small.

EXPERIMENTAL.

A complete examination of the chief constituents of the two straws used in this investigation, namely oat straw and rye straw, was first made; and it will be convenient now to discuss the former, group by group.

The analytical figures obtained were as follows.

Oat straw.

						%
Ash	...	...	...	...	...	7.07
Total furfuraldehyde yield	...	...	...	...	...	16.08
Carbon dioxide yield	...	...	...	...	...	1.21
Calcium pectate yield	...	...	...	...	...	1.10
Cellulose (Cross and Bevan)	...	...	...	...	...	53.14
Furfuraldehyde from cellulose	...	...	...	...	...	5.99
Lignin	...	...	...	...	...	18.54
Protein	...	...	...	...	...	1.8

Determination of hemicellulose content.

It is well known that the hemicelluloses contain considerable quantities of uronic acids and, as mentioned above, these acids yield a certain amount of furfuraldehyde. The total furfuraldehyde may be derived, therefore, from the pentose and uronic groups in the hemicelluloses, from the pectin, and from the rather obscure substances in the Cross and Bevan cellulose fraction. It is possible from the data above to disentangle these various sources, and to present an ordered balance sheet of the constituents.

Let *A* represent total furfuraldehyde yield.

„ *B* „ furfuraldehyde from cellulose fraction.

„ *C* „ furfuraldehyde from pectin (19.5 % on calcium pectate).

„ *D* „ furfuraldehyde from total uronic acid anhydride (16.66 %).

„ *E* „ furfuraldehyde from uronic acid anhydride in pectin (11.6 % on calcium pectate).

From this (*C-E*) represents furfuraldehyde from the arabinose group in pectin. Then the figure for furfuraldehyde arising from the pentose groups of the hemicelluloses present in 100 g. straw is given by

$$[A - \{B + D + (C - E)\}]$$

which in the case of this straw is

$$[16.08 - \{5.99 + 0.81 + (0.21 - 0.13)\}] = [16.08 - 6.88] = 9.2 \text{ \%}.$$

In order that this might be interpreted in terms of hemicellulose it was necessary next to determine the actual furfuraldehyde yield, since, as mentioned above, it is not possible to assume that only pentose groups are present. The method of extraction was suggested by Norris and Preece [private communication]. 100 g. of the finely powdered straw were extracted on a boiling water-bath with 1 litre 0.5 % ammonium oxalate twice for a period of 4 hours each to remove the pectic substances, after which it was well washed with boiling water. This was followed by two treatments for 2 hours each with a litre of 55 % alcohol containing 1 % NaOH, with the object of extracting the more readily soluble lignin. After washing by a short treatment with 55 % alcohol alone, the extraction of hemicelluloses was effected by four treatments with 4 % NaOH, the first for 4 hours, and the others for 2 hours

each. The combined alkaline extracts of the hemicelluloses were filtered through packed glass wool, and acidified with glacial acetic acid. A colloidal solution was obtained, from which the mixed hemicellulose product was precipitated by addition of alcohol. After filtering, the precipitate was washed with acetic acid and alcohol mixture, and redissolved in dilute NaOH. Re-precipitation was carried out as before by acidification with acetic acid and addition of alcohol. The product was dried with alcohol and ether.

The yields of furfuraldehyde and CO₂ were determined on this mixed product.

		%
Furfuraldehyde yield on ash-free basis	...	43.0
CO ₂ yield on ash-free basis		3.9
Uronic acid anhydride		15.6
Furfuraldehyde due to uronic groups	...	2.6

By difference, the furfuraldehyde due to pentose units of hemicellulose = 40.4 %. Since it was determined above that the amount of furfuraldehyde arising from the pentose groups of the hemicelluloses present in 100 g. straw was 9.20, and the pentose units in the hemicelluloses themselves yield 40.4 % furfuraldehyde, it is clear that an approximation for the actual hemicellulose content is given by the quotient, namely 22.8 %.

The nature of the hemicelluloses present.

The nature of the hemicelluloses present in this mixture was next investigated. The mixed product was separated into two portions, hemicellulose A and hemicellulose B according to the method proposed by O'Dwyer [1926]. There is reason to suppose that these also are not individual entities but mixtures. The mixed product was dissolved in NaOH and precipitated by large quantities of glacial acetic acid. The excess of acid results in the hemicellulose A fraction being precipitated in an easily filterable form. To the filtrate was added alcohol thereby precipitating hemicellulose B. Purification was carried out in each case by repetition of the same procedure.

Oat straw.

	Hemicellulose A	Hemicellulose B
	%	%
Furfuraldehyde yield on ash-free basis	42.9	43.3
CO ₂ yield on ash-free basis	2.7	7.95
Uronic acid anhydride	10.8	31.80
Furfuraldehyde due to uronic groups	0.65	1.90
Furfuraldehyde due to pentose groups...	42.25	41.5

The amount of the former obtained was much in excess of the latter, indeed from the uronic content of the mixture it would seem that the proportion lies between three and four to one.

Hydrolysis of each was carried out with 2 % H₂SO₄ for 6 hours at 100°,

the liquid being then neutralised with barium carbonate and the filtrate concentrated under reduced pressure to a thin syrup.

The sugars tested for were mannose as hydrazone, arabinose as the diphenylosazone, xylose as the cadmium xylonobromide salt, and galactose as mucic acid. It was not possible in either case to characterise the uronic acid present owing to the small quantity available.

Preparation	Mannose	Arabinose	Xylose	Galactose
Hemicellulose A	-	+	+	+
Hemicellulose B	-	+	-	-

It is probable therefore that hemicellulose A consists of about 11 % uronic acid anhydride, 79 % arabinose and xylose calculated as anhydroarabinose, and 10 % anhydrogalactose, while hemicellulose B yielding about the same percentage of furfuraldehyde contains 32 % uronic acid anhydride, and 68 % arabinose.

The nature and quantity of the furfuraldehyde-yielding substances in the Cross and Bevan cellulose.

It was next necessary to investigate the nature of the furfuraldehyde-yielding groups in the Cross and Bevan cellulose product. The cellulose fraction of straw isolated by this means is not unique in that it yields a considerable amount of furfuraldehyde, in this case over 11 %, since practically all Cross and Bevan celluloses appear to do this whatever their source. It has been customary arbitrarily to calculate the results as "pentosans" and to subtract this from the percentage of Cross and Bevan cellulose. This procedure has been criticised on the grounds that the furfuraldehyde may arise from sources other than pentosans, such as oxidation products formed from cellulose during the process of chlorination. This possibility was first examined.

The action of practically every oxidising agent on cellulose is the production of oxycelluloses, to a greater or less extent. This rather vague group has received the attention of many investigators, since the production of oxycellulose in the treatment of fibres and fabrics is accompanied by a loss of strength. Oxycelluloses contain an aldehydic group as evidenced by the reduction of Fehling's solution, and the formation of osazones. Further, they yield furfuraldehyde on distillation with 12 % HCl, the amount varying with the degree of oxidation employed in their preparation. More recently it has been shown by Heuser and Stöckigt [1922], and Hibbert and Parsons [1925] that carbon dioxide is evolved on suitable acid treatment, indicating the presence of glycuronic acid groups. This would explain the production of furfuraldehyde and led the workers named above to suggest that the process of production of oxycelluloses is accompanied by the progressive formation of uronic groups. It has not, however, been shown that the furfuraldehyde from oxycelluloses arises only from these uronic groups, and to throw some light on this point the yields of furfuraldehyde and CO₂ from the same preparations were determined.

Preparation	Source	Oxidising agent	Copper number	Furfuraldehyde yield %	CO ₂ yield %	Uronic acid anhydride %	Furfuraldehyde due to uronic acid anhydride %
α -Oxycellulose	Cotton-wool	KClO ₃ + H ₂ SO ₄	4.8	0.80	0.62	2.48	0.41
β -	"	HNO ₃	9.6	0.65	1.1	4.4	0.72
γ -	"	H ₂ SO ₄ on β	9.4	0.40	0.66	2.63	0.42
δ -	"	NaOH to β filtrate	14.0	1.18	1.36	5.44	0.91

It will be seen that the furfuraldehyde due to the uronic acid is approximately the total furfuraldehyde given by the preparation within the limits of error of the latter estimation, so that it is clear that the sole result of oxidation is the production of uronic groups.

It was next important to determine the extent of the oxidation which takes place in the Cross and Bevan chlorination. To this end samples of cotton-wool and filter-paper were chlorinated for three periods of 12, 7 and 5 minutes, being treated with sulphite in the intervals as described previously. The yields of furfuraldehyde of the original sample and of the final chlorination product were determined. This process of chlorination results, therefore, in only a very small production of uronic groups, and the criticism that the furfuraldehyde-yielding substances found with the Cross and Bevan cellulose fractions are due to oxidation products is not justified.

Material	Recovery after chlorination, %	Furfurald. before chlorination, %	Furfurald. after chlorination, %
Cotton-wool	98.24	0.66	0.71
Filter-paper, Whatman No. 2	92.58	0.18	0.21
Filter-paper, Whatman No. 41	98.74	0.13	0.23

However, the precise nature of these substances in the cellulose is a difficult problem to settle. A determination of the yield of CO₂ from the chlorination product at once shows that it is not a hemicellulose similar to the free hemicelluloses isolated, for the content of uronic acid anhydride is extremely low.

	%
Furfuraldehyde yield of Cross and Bevan cellulose	11.32
CO ₂ yield of Cross and Bevan cellulose	0.22
Uronic acid anhydride	0.88
Furfuraldehyde due to uronic acid anhydride	0.15

Two weighed portions of the chlorination product were extracted for 4 hours at 100° with 4 % NaOH. The alkali was partially neutralised and filtered. After washing, one portion was used for the determination of the yield of furfuraldehyde, and the other for that of CO₂.

	%
Furfuraldehyde yield of alkali-extracted cellulose	9.2
CO ₂ yield of alkali-extracted cellulose	0.23

From the fact that the furfuraldehyde yield was lowered without affecting

that of CO_2 , it is to be supposed that the pentose groups yielding furfuraldehyde are not combined with the uronic acid, though this supposition involves the doubtful assumption that this substance in the cellulose is homogeneous. It would seem not unlikely from the evidence that this small amount of CO_2 arises from a fraction of natural oxycellulose present, together with that formed by oxidation during the chlorination process. The alternative is that there is a small amount of hemicellulose similar in type to hemicellulose A and B.

It is not possible to prepare any large amount of the chlorinated product, so that any attempt at a preparation of the main furfuraldehyde-yielding substances in it is out of the question. Since the presence of hexosans in the Cross and Bevan cellulose fraction has been reported in the case of certain woods, a portion was boiled with 1 % sulphuric acid for several hours. After neutralisation the extract was tested for mannose and galactose without result. The pentose present appeared to be xylose. There is reason to suppose that very little is removed by this acid hydrolysis, and in consequence the result is not entirely conclusive. However, it seems not improbable that it is a xylan intimately associated with the cellulose which is responsible for this yield of furfuraldehyde. Irvine and Hirst [1924] have shown that esparto cellulose contains 81.5 % normal cellulose and 18.5 % xylan, while Heuser and Haug [1918] state that the cellulose from a cereal straw (type unspecified) contained as much as 22.34 % xylan. In the straw under investigation the pentose present calculated as anhydroxylose amounts to 17.35 % of the cellulose product.

The relationship of this xylan to the true cellulose has been a matter of considerable controversy, since the xylan appears to be so intimately associated that removal is a matter of considerable difficulty. Two extractions were carried out to investigate this point. In the first, 100 g. straw were treated in a boiling water-bath with 1 litre 0.5 % ammonium oxalate twice for a period of 1 hour each, to remove pectin. This was followed by four treatments of an hour with 4 % NaOH , and finally with water to ensure that all the alkali had been removed. The following analytical figures were obtained on this residue:

	C. and B. cellulose %	Total furfurald. %	Furfurald. on 100 g. C. and B. cellulose g.	Furfurald. on cellulose in 100 g. material g.	Carbon dioxide yield %	Uronic acid %
Oat straw	53.14	16.08	11.32	5.99	1.21	4.84
Residue	91.68	9.09	7.83	7.18	0.46	1.84
100 g. original straw	50.3	5.0	—	3.9	0.25	1.00

It was not possible to determine the actual loss during this treatment, since the alkaline extracts have to be filtered on cloths, during which some losses occur. It is, however, possible approximately to calculate this. The calculation depends on the assumption that there is no loss of true cellulose in this extraction. The total amount of true cellulose originally present was 53.14 %,

less the associated xylan which amounted to $(5.99 \times 1.55) \%$, 1.55 being the factor for obtaining anhydroxylose from the yield of furfuraldehyde. In the course of the extraction a certain portion of the xylan associated with the cellulose is removed. The amount remaining may be determined from the ratio of furfuraldehyde given by 100 g. Cross and Bevan cellulose after extraction to that given by 100 g. original product. This amounts to

$$\left[(5.99 \times 1.55) \times \frac{7.83}{11.32} \right].$$

The total residual straw may be designated by Q , 91.68 % of which is Cross and Bevan cellulose, composed of the true cellulose present originally in 100 g. straw, together with the residual associated xylan. Expressed in figures, this is

$$91.68 \frac{Q}{100} = \left[53.14 - (5.99 \times 1.55) + \left\{ \frac{(5.99 \times 1.55 \times 7.83)}{11.32} \right\} \right]$$

$$Q = 54.8 \%.$$

The loss during extraction is therefore 45.2 %. It is then possible from this to obtain the analytical figures of the residue calculated back on 100 g. of original straw. These are given in the third horizontal row of the table above. While the furfuraldehyde yield from groups unassociated with the cellulose has decreased from 10.08 % to 1.1 %, a loss of nearly 90 %, that from the xylan has fallen from 5.99 % to 3.9 %, a loss of only 35 %.

A second extraction which was carried out was very much more drastic. 100 g. of dried straw, finely ground, was treated on a boiling water-bath with, in turn, 1 litre 0.5 % ammonium oxalate for two periods of 4 hours each, 4 % NaOH for four periods of 4 hours each, and finally with water for two periods of 2 hours. The residue after drying gave the following analytical figures:

	C. and B. cellulose %	Total furfurald. %	Furfurald. on 100 g. C. and B. cellulose g.	Furfurald. on cellulose in 100 g. material g.
Oat straw	53.14	16.08	11.32	5.99
Residue	94.41	3.05	3.07	2.90
100 g. original straw	48.05	1.55	—	1.48

The loss during extraction was determined as described above from the expression

$$94.41 \frac{Q'}{100} = \left[53.14 - (5.99 \times 1.55) + \left\{ \frac{(5.99 \times 1.55 \times 3.0)}{11.32} \right\} \right]$$

$$Q' = 49.1 \%.$$

The loss was therefore 50.9 % indicating the removal of all but a trace of the furfuraldehyde groups unassociated with the cellulose, while the associated xylan sustained a loss of only 75 %.

In this property of great resistance to extraction the xylan associated with the cellulose of this straw is in no wise different from that of other straws and woods as noted by various investigators. Heuser and Haug [1918], when

employing reasonable methods of extraction, found it impossible completely to free from xylan the cellulose from a cereal straw. Six extractions with 6 % NaOH at 100° only resulted in a loss of 80 %, while the residue after treatment with NaOH under pressure at 140–150° retained about 30 % of the xylan. By very drastic treatment with 17 % NaOH in the cold it is possible to remove practically all of the furfuraldehyde-yielding substances, but at the same time the β - and γ -cellulose fractions are also removed.

The relationship of this associated xylan to the cellulose is a question upon which it is impossible to come to a decision. Cross and Bevan originally viewed as cellulose all the non-nitrogenous structural matter of plants, stating that there were many different forms of cellulose, both physical and chemical. It gradually became recognised that the cotton fibre was alone in consisting of cellulose almost solely, cellulose in this sense being a chemical entity built up of anhydroglucose groups. Other plant tissues were stated to be composed of a cellulosic group in attachment with lignin as "lignocelluloses," pectin as "pectocelluloses," hexosans as, for example, the mannocellulose of Schulze, with pentosans, and with other groups. Wislicenus and Kleinstück [1910] were the forerunners of the modern adsorption theory. Though applied by them to the process of lignification, it applies to all groups, since they viewed lignin as being the sum total of the hydrosols of the cell sap deposited on the cellulosic membranes by adsorption. Modern methods of attack have thrown more light on this problem. X-ray analysis employed by Hertzog and Jancke [1920] indicates that the cellulose from many plant resources is identical, while Irvine and Hirst [1924] by their methylation process have shown that the cellulose from esparto is chemically identical with cotton cellulose. Most investigators are now agreed in viewing cellulose as a large aggregate made up of many units each comparatively small in size, containing only a few anhydroglucose groups. These units are presumed to be held together by secondary valency, interference with which results in the alteration of the physical state and the degree of adsorption possible. It is also conceivable that other units, such as pentosans which have been formed during development in close association with the cellulose, may be present in the aggregate, being held by secondary valency. The type of these units found will depend on the conditions obtaining during growth, but the amount found may bear no relationship to the amount really present, since it depends entirely on the method of purification to which the cellulosic tissue has been subjected. Two methods of association are involved in this theory, adsorption and casual linkage by secondary or residual valency. Further, it is not impossible that the difficulty of extraction of these associated substances may also be in part a mechanical one. Furfuraldehyde-yielding substances appear very early in the development of a tissue and it is possible that in thickening cellulose may be deposited on the pentosan material. The middle lamella, originally pectic in nature, may be converted by decarboxylation into pentosan, which would be entirely "occluded" in the cellulose of the walls. Such "occluded" material,

even if in itself quite soluble, would be difficult of extraction until the cellulosic barrier had been ruptured or physically altered by drastic treatment such as gelatinisation by alkali. It is claimed that this may be in part a contributory factor in the apparent resistance of these substances associated with the cellulose aggregate.

Having thus considered the nature of the furfuraldehyde-yielding substances of this straw, it is possible to present a more rational balance sheet of the various constituents. The methods which must be followed to obtain these figures obviously prevent any claim of absolute accuracy, but it is claimed that they are a close approximation. It is certain that there is some overlapping, though it is not easy to see where this occurs unless it is in the lignin fraction. The method of Ost and Wilkening [1910] involving the use of 72 % H_2SO_4 gave a mean figure of 18.54 %, while the alkaline extraction method due to Mehta [1925] gave a figure for "lignic acid" of only 8.43 %. It is possible that an unduly high figure for lignin may be obtained for reasons connected with the structure of the tissue. It would not be unreasonable to picture a layer of lignin, either adsorbed, or attached by secondary valency and completely overlying a unit of cellulose. By the acid method the whole of this fragment might be returned as lignin even though the resistant lignin coating was but a fraction of the whole. The analyses are not complete, for no attempt has been made to determine accurately such groups as tannins, starch, and free sugars, since these were not of major importance in the fermentation studies to follow. Roughly, however, these last do not together account for more than 2 % of the whole. This method of evaluation of the furfuraldehyde-yielding constituents is applicable generally, and has been successfully applied in the case of certain woods.

It is now possible to summarise the various interpretations of the analyses of this straw originally quoted on p. 1356.

Composition of oat straw.

	%
Ash	7.07
Free hemicellulose	22.8
"Pure" cellulose	43.8
Xylan associated with cellulose	9.3
Pectin (calcium pectate yield)	1.1
Protein	1.8
Lignin (72 % H_2SO_4)	18.5

The same procedure was followed throughout in the case of rye straw.

Rye straw.

	%
Ash	3.46
Total furfuraldehyde yield	17.05
Carbon dioxide yield	1.15

Rye straw (continued).

	%
Calcium pectate yield	0.34
Cellulose (Cross and Bevan)	55.27
Furfuraldehyde from cellulose	5.64
Lignin	19.5
Protein	1.9

Furfuraldehyde arising from the pentose groups of the hemicelluloses present in 100 g. straw is given by

$$[A - \{B + D + (C - E)\}],$$

whence

$$[17.05 - \{5.64 + 0.77 + (0.07 - 0.04)\}] = [17.05 - 6.44] = 10.61 \text{ \%}.$$

Mixed hemicellulose preparation.

	%
Furfuraldehyde yield on ash-free basis ...	34.0
CO ₂ yield on ash-free basis	3.3
Uronic acid anhydride	13.2
Furfuraldehyde due to uronic groups ...	2.2

By difference, furfuraldehyde due to pentose units of hemicellulose 31.8 %.
The free hemicellulose content of this straw is therefore approximately 33.4 %.

Purification of the mixed hemicellulose product was carried out.

Rye straw.

	Hemicellulose A %	Hemicellulose B %
Furfuraldehyde yield on ash-free basis...	32.7	37.1
CO ₂ yield on ash-free basis	1.29	7.1
Uronic acid anhydride	5.16	28.4
Furfuraldehyde due to uronic groups ...	0.86	4.7
Furfuraldehyde due to pentose groups...	31.8	32.4

The nature of the sugars present in these products was not determined. Hemicellulose A contains however approximately 5 % uronic acid anhydride, 60 % anhydropentose (calculated as arabinose) and 35 % anhydrohexose. Hemicellulose B is composed of approximately 28.5 % uronic acid anhydride, 60.5 % anhydropentose (calculated as arabinose) and 11 % anhydrohexose.

The furfuraldehyde-yielding substance associated with the cellulose appeared in this case also to be xylan. In amount it is present to the extent of 15.82 % of the Cross and Bevan cellulose fraction, or 8.74 % of the straw. As it will be seen from the analytical figures quoted below, the xylan was even more difficult of extraction than that of oat straw. Two series of extractions were carried out, the first with 4 % NaOH for four periods of 4 hours each, and the second similarly but preceded by two treatments of 2 hours each with 1 % NaOH in 55 % alcohol.

	C. and B. cellulose %	Total furfurald. yield %	Furfurald. on 100 g. C. and B. cellulose g.	Furfurald. on cellulose in 100 g. material g.
Rye straw	55.27	17.05	10.21	5.64
Extraction I	90.01	5.73	5.57	5.01
Extraction II	91.56	5.50	5.57	5.10

By calculation the loss in the case of extraction I was approximately 43 %, and in the case of extraction II, 44 %. It was possible therefore to determine the true loss of each group as determined on 100 g. of the original material.

	C. and B. cellulose %	Total furfurald. yield %	Furfurald. on C. and B. cellulose in 100 g. material g.
Preparation I	51.30	3.27	2.86
Preparation II	51.24	3.07	2.85

In the case of the more drastic extraction, the furfuraldehyde yield from groups unassociated with cellulose has fallen from 11.41 to 0.22, a loss of 98 %; while that of the xylan has fallen from 5.64 to 2.85, a loss of only 50 %. The xylan associated with the cellulose of oat straw sustained a loss of 75 % under the same treatment. There is obviously therefore a considerable difference either in the physical condition or method of linkage of this substance in these two straws, which must be considered later when the availability to micro-organisms is being discussed.

It will be convenient now to re-state the results obtained for the composition of this straw. Overlapping was again evident, though not possible to place, unless again it occurred in the lignin fraction.

Composition of rye straw.

	%
Ash	3.46
Free hemicellulose	33.40
"Pure" cellulose	46.53
Xylan associated with cellulose	8.34
Pectin (calcium pectate yield)	0.34
Protein	1.9
Lignin (72 % H_2SO_4)	19.50

SUMMARY.

1. Figures quoted for "pentosan" obtained by use of the Kröber factor on total furfuraldehyde yield are unreliable because of the uronic and hexose groups in the hemicelluloses, and the furfuraldehyde-yielding groups intimately associated with the Cross and Bevan cellulose fraction.

2. If an adjustment is made for these and for the pectin present, it is possible to obtain a satisfactory figure for furfuraldehyde due only to the pentose units of the hemicelluloses.

3. An approximation for the actual content of hemicelluloses in the

material may then be obtained by determining the furfuraldehyde yield from the pentose groups in a mixed hemicellulose product obtained by alkaline extraction.

4. Oat straw is shown to contain approximately 22.8 % free hemicelluloses, being a hemicellulose A consisting of about 11 % uronic acid anhydride, 79 % arabinose and xylose (expressed as anhydro-arabinose) and 10 % anhydro-galactose, and a hemicellulose B yielding about the same percentage of furfuraldehyde but containing 32 % uronic acid anhydride and 68 % arabinose.

5. The furfuraldehyde-yielding substance associated with the Cross and Bevan cellulose fraction in both oats and rye is shown not to consist of oxidation products arising from the chlorination process, but to be due to xylan free from uronic groups. This xylan is extremely resistant to extraction and is presumably held by the cellulose aggregate by secondary valency. It is suggested that the difficulty of extraction may be, in part at least, a mechanical phenomenon, a portion of the pentose being "occluded" during development.

6. Rye straw is shown to contain about 33 % of free hemicelluloses, the hemicellulose A fraction being built up of approximately 5 % uronic acid anhydride, 60 % anhydropentose, and 35 % anhydrohexose, while the hemicellulose B fraction contains roughly 29 % uronic acid anhydride, 60 % anhydropentose, and 11 % anhydrohexose.

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