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ROTHAMSTED RESEARCH

Report for 1935

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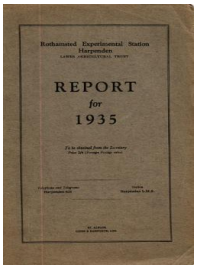

The Biochemistry Section , 1933-1935

Rothamsted Research

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depends on an optimum relationship between viscosity and elasticity modulus, and the latter, although not yet fully understood, is related to the way in which viscosity varies with stress. The bearing of these properties on bread making quality is being investigated. Doughs which tear easily, and consequently give bread with ragged crust and bad texture, are said to be " short." This property of " shortness," and the relationships between the breaking and the flowing of plastic materials, are being studied. Brittleness, which is disadvantageous in the flour-dough, is beneficial in the soil crumb, and the methods developed for the study of the dough are now being modified for application to the soil. The same principles are being applied at the National Dairy Institute for measuring the elastic and plastic properties of cheeses.

Flour doughs show a definite structure which is broken down on kneading, and re-establishes itself on standing. This property is fairly common and is called " thixotropy " (see also p. 64).

THE BIOCHEMISTRY SECTION 1933-1935

A. G. NORMAN

The work of this section consists in a study of the composition and decomposition of plant constituents, particular attention being given for the present to the carbohydrates.

METHODS OF ANALYSIS OF PLANTS

The conventional methods of analysis of agricultural materials give a very imperfect picture of the composition of a plant, being restricted usually to such determinations as ash, total nitrogen, (calculated as crude protein), ether-soluble material, and crude fibre, the difference of the sum of these from 100 being regarded as " soluble carbohydrates." Before any extended study of the composition of crops could be attempted, a more detailed and searching system of analyses had to be found to cover the carbohydrate constituents. This involved particularly the testing of methods for the determination of those structural constituents which are most inadequately represented by the crude fibre figures. The main structural constituents, cellulose, lignin and hemicelluloses, together account for the major part of any mature tissue. Existing methods have not been found to be generally applicable without modification.

1. *Cellulose.* The cellulosic framework of plant tissues is determined after removal of all other constituents. In fact, however, lignin is the most difficult component to remove. The Cross and Bevan procedure of alternate exposure to gaseous chlorine and extraction with boiling sodium sulphite is the basis of nearly all methods, the lignin thereby passing into solution as sulphonic derivatives. The conditions under which the chlorination may be carried out in dilute hypochlorite solution, have been examined,

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and a more rapid and convenient method developed (1). The cellulose of plants and wood differs from that of the cotton hair in that it is not solely composed of glucose units, but contains also polysaccharides of other sugars, very intimately associated. These polysaccharides, which have been termed "cellulosans" (2) are more susceptible to hydrolytic agents, and more soluble in alkalis, than the true cellulose portion of the aggregate, so that all treatments other than with neutral solutions must be avoided if the integrity of the natural cellulose is to be preserved. The method devised is also suitable for making large-scale preparations of plant celluloses which was not possible previously. In most cases the associated cellulosan is xylan, which may be determined by the yield of furfuraldehyde from the isolated cellulose.

2. *Lignin.* The basis of the determination of lignin is the resistance of this substance to strong acids, in which cellulose and other carbohydrates pass into solution, subsequently to be hydrolysed. Existing methods, devised mainly for woods, have been shown to be inaccurate and quite inapplicable to agricultural materials, which unlike woods are often high in nitrogen. Two major and interacting sources of error have been shown to exist, due to the presence of pentoses and proteins. Pentoses or pentosans on contact with strong acids slowly give furfuraldehyde which in the absence of lignin condenses to form a black insoluble residue weighed as lignin (3) or in the presence of lignin unites with it to give a stable ligno-furfuran resin, thereby increasing the apparent lignin content. This disturbance may be minimised by shortening the period of contact with the strong acid, or by a hydrolytic pre-treatment (4). The error introduced by the presence of protein is at present more obscure and has not been wholly overcome. Proteins themselves or protein degradation products give no residue on treatment with strong acid but if added to a lignified material increase the apparent lignin content (5). Small quantities of protein cause a greater disturbance proportionately than do larger amounts, the error being due to the linkage with lignin of protein fission products of varying size. Acid pre-treatment results in a substantial reduction of the interference in most cases, which cannot be allowed for by calculating the nitrogen content of the lignin residue as protein and deducting. Because of these sources of error in the lignin determination the figures generally quoted for the lignin content of plant materials are in most cases too high.

3. *Hemicelluloses.* A satisfactory method for the routine determination of hemicelluloses has not yet been devised. Extraction methods that have been proposed are incapable of distinguishing between the true encrusting polyuronide hemicelluloses, and the cellulosan fraction of the cellulose, which has very similar properties.

(1) A. G. Norman and S. H. Jenkins—"A New Method for the Determination of Cellulose based upon Observations on the Removal of Lignin and other Encrusting Materials." Biochem. Journ., 1933, Vol. XXVII, pp. 818-831.
(2) L. F. Hawley and A. G. Norman—"The Differentiation of Hemicelluloses." Ind. and Eng. Chem., 1932, Vol. XXIV, pp. 1190-1195.
(3) A. G. Norman and S. H. Jenkins—"Lignin Content of Cellulose Products." Nature, 1933, Vol. CXXXI, p. 729.
(4) A. G. Norman and S. H. Jenkins—"The Determination of Lignin, I. Errors introduced by the Presence of Certain Carbohydrates." Biochem. Journ., 1934, Vol. XXVIII, pp. 2147-2159.
(5) A. G. Norman and S. H. Jenkins—"The Determination of Lignin, II. Errors Introduced by the Presence of Proteins." Biochem. Journ., 1934, Vol. XXVIII, pp. 2160-2168.

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For the present and for comparative purposes, reliance is placed upon the yield of furfuraldehyde from the pentose and uronic acid groupings as a measure of the amount of encrusting hemicelluloses, this figure being arrived at by the difference between the total furfuraldehyde yield and that from the cellulosan groups in the cellulose.

The analyses mentioned above taken together provide a full picture of the structural constituents of any plant material and permit a detailed examination of the " crude fibre " determination, so much employed in agricultural analysis; by their aid it has been possible to show what exactly this fraction represents(6). The crude fibre figure may be very misleading since no constant or definite proportions of the structural constituents are included. The cellulose is partially attacked so that only 60 to 80 per cent. remains, and the lignin extensively removed by the acid and alkaline treatments given. Much variation may be found in the lignin content of crude fibre fractions. Since the presence of lignin exercises a direct effect on the digestibility of the material, any empirical method should include all the lignin. For certain purposes a simple acid hydrolysis would supply more reliable information than the crude fibre determination.

The Composition of Crops

Suitable methods having been devised a study of the composition of certain crop materials was commenced along the lines of a preliminary investigation carried out in 1930 on barley(7). Samples are cut at frequent intervals during growth so that the developmental changes may be followed. So far the investigations have been confined to winter wheat, and rye grass (Western wolths). The latter revealed several interesting features which are to be the subject of future examination. A high percentage of cold water-soluble material was found in this grass, at one stage nearly 55 per cent., and at maturity almost 40 per cent., the bulk of which is accounted for by a fructosan, or levan. At one stage the fructosan content was found to be 35 per cent. but as the grass approached maturity the amount of this constituent fell sharply while at the same time the cellulose increased. Neither the protein nor the lignin contents changed as widely as expected, and it is clear that a relatively small change in the amount of lignin present is responsible for the considerable decrease in digestibility that accompanies maturity. On drying for hay in the usual manner, the losses appeared to be of the order of 10 to 15 per cent., much of which could be accounted for by the disappearance of fructosan. Preparations of this fructosan have been found to be more susceptible to acid hydrolysis than any other polysaccharide, being completely broken down to fructose by heating with oxalic acid as dilute as 0.05 per

(6) A. G. Norman—" The Composition of Crude Fibre." Journ. Agric. Sci., 1935, Vol. XXIV pp. 529-540.

(7) A. G. Norman—" A Preliminary Investigation of the Development of Structural Constituents in the Barley Plant." Journ. Agri. Sci., 1933, Vol. XXIII, pp. 216-227.

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cent. for one hour. Fructosans have also been found in wheat, though not to such an extent; apparently they have an important rôle in the metabolism of the Gramineae as a temporary carbohydrate reserve.

The Plant Cell-wall

Accompanying these applied studies of plant composition, some more fundamental work on the nature and inter-relationship of the cell-wall constituents has been undertaken, mainly on cereal straws and fibre plants.

1. *Cellulose.* It has been stated before that most plant celluloses are very different from cotton cellulose in that they are aggregates of pure cellulose and an associated polysaccharide or cellulosan, which is often xylan. Many plant celluloses have been isolated and their properties and stability studied with a view to obtaining information as to the method of association of the cellulosan, and its influence on the properties of the whole. The results are not inconsistent with the view that the cellulosan molecules, though much shorter in length, are oriented like the long cellulose chains and participate in the micellae, being retained by the same type of secondary valency forces. Heat drying liberates a water-soluble fraction, mainly of cellulosan, from a natural cellulose previously unaffected by water, and this phenomenon may be repeated many times. Any attack on the cellulosan such as acid hydrolysis or alkaline extraction is accompanied by a partial attack on the cellulose so that no hard and fast line can be drawn between the two groups. Solution and reprecipitation from a cellulose solvent, by destroying the orientation of the molecules, renders the cellulosans easily soluble. To obtain additional evidence on the distribution of the xylan in such celluloses, experiments have been carried out on certain vegetable fibres in conjunction with Mr. W. T. Astbury, of the University of Leeds, using X-ray methods. The removal of xylan progressively from manilla hemp cellulose was accompanied by an improvement in the X-ray picture indicating a more perfect crystallographical state. When freed from xylan, the diagram was almost indistinguishable from those of ramie and Italian hemp, both celluloses which are naturally very low in xylan(8). The diagram for isolated xylan has also been obtained by the preparation of thin films. The X-ray evidence is in accord with the view that the xylan molecules are iso-structural with those of the true cellulose chains.

In a study of the composition of vegetable fibres of many types, it was found that these fall into two well-defined groups according to whether the cellulose of the fibre was high or very low in xylan.(9) The group high in xylan includes the coarser fibres, such as jute, manilla hemp, and sisal, all of which contain also appreciable amounts of lignin and encrusting substances. The second group, low in xylan, includes the high-grade fibres such as flax,

(8) W. T. Astbury, R. D. Preston and A. G. Norman—"X-Ray Examination of the Effect of Removing Non-Cellulosic Constituents from Vegetable Fibres." Nature, 1935, Vol. CXXXVI, p. 391.

(9) A. G. Norman—"The Composition of some Vegetable Fibres, with particular reference to Jute." Biochem. Journ., 1936, Vol. XXX (in the press).

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ramie and hemp. No direct relationship between xylan content and quality could be found in a wide range of jute samples.

2. *Lignin.* Arising out of the observation reported previously that lignin condenses with furfuraldehyde in the presence of strong acid, to give a complex of the nature of a phenolic resin, similar resins with a number of other aldehydes have been prepared. Imperial Chemical Industries have moulded the lignin-formaldehyde resin under high pressure. Phenol may further be condensed with the lignin-aldehyde complex with a resulting modification of properties. Lignin resins with acetaldehyde or higher aldehydes have not yet been tested commercially.

Considerable attention has been given to the delignification of plant materials by means of solvents. For many research purposes it would be desirable to achieve a complete removal of lignin without at the same time bringing about hydrolysis or degradation of the carbohydrates. Solvents such as pyridine, dioxan, alcohol, etc., have been tried, with, and without, previous chlorination, but so far only partial success has been achieved. The removal of lignin with alcoholic soda has been investigated since this has been proposed as a pretreatment to the preparation of the hemicelluloses, but neither cold nor hot is this an effective delignifying agent.(10)

3. *Hemicelluloses.* The encrusting polyuronide hemicelluloses are an important group in many agricultural materials; in straw, for example, they form the second largest constituent. The study of their composition is beset with difficulties, and all preparations are more or less seriously contaminated with lignin and cellulosan. To reduce the former, it has been customary to effect a partial delignification with alcoholic soda, but this has been shown to have at the same time a serious effect on the hemicelluloses, and particularly on those of immature tissues.(10) A study of the removal of lignin and hemicelluloses from the cell-wall by alternate chlorination and sulphite extraction has thrown some light on the condition of the hemicelluloses *in situ.*(11) It seems likely that there is some form of close association, probably amounting to actual chemical combination, between the hemicelluloses and lignin, since the former cannot be extracted unless a pretreatment (such as chlorination) is given capable of rupturing the linkage, or unless a solvent (such as alkali) is used which at the same time dissolves the lignin. The classical conception of the existence of a "lignocellulose" has had to be abandoned in view of modern work on the structure of cellulose, and it now appears that a lignin-hemicellulose complex may be substituted for it. Possibly both groups occur in two conditions, combined and free.

Decomposition of Plant Materials

The limitations of the lignin determination now being understood, it was thought worth while to take up again the question of the biological decomposition of lignin upon which subject many

(10) A. G. Norman—"The Hemicelluloses. I. Alcoholic Sodium Hydroxide as a Pretreatment to Extraction." Biochem. Journ., 1935, Vol. XXIX, pp. 945-952.

(11) A. G. Norman and (in part) J. G. Shrikhande—"The Hemicelluloses, II. The Association of Hemicelluloses with Lignin." Biochem. Journ., 1935, Vol. XXIX, pp. 2259-2266.

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conflicting opinions have been expressed. Studies on the availability of lignin involve analyses from time to time on materials that are changing in composition. It is very significant that changes are especially marked in those two groupings which contribute so largely to errors in the lignin determination, for pentoses are rapidly fermented away and proteins synthesised under normal conditions of ærobic decomposition. To some extent these two errors are compensatory and their effect varies widely with the nature of the material and period of decomposition. Previous observations have been re-examined in the light of these facts.(12) The ærobic decomposition of lignin in straw has been studied and determinations made over a period of eighteen months by four different methods, all of which show losses of 40-50 per cent. of the lignin in the first year and 50-60 per cent. in eighteen months.(13) Lignin is certainly not so resistant to biological attack as has sometimes been claimed, but being the most resistant plant constituent tends to accumulate. Highly decomposed organic residues composed largely of lignin and protein are readily susceptible to oxidation, and this property is being investigated in humic residues from various sources.

Certain of the oak timbers of Rothamsted House have been very extensively attacked by the Death-watch Beetle, and on their replacement the opportunity was taken of analysing samples of the decomposed wood and comparing them with the sound wood from the same source. The results leave no doubt that the main constituent removed by the larvæ was the cellulose, and in so far as it was possible to form any estimate, the total loss suffered by the wood was in the region of one third.(14)

Other Investigations. The oxidation of amino acids with hypochlorite has been studied in detail, and the route of the reaction and products determined. Glycine gives rise to CO_2, water and gaseous N, through the intermediate formation of HCN, which is subsequently hydrolysed to formic acid and ammonia, both then being completely oxidised. The rate of the reaction is enormously affected by the pH of the mixture, being most rapid in the region of pH 7-9.(15) When extended to higher amino acids, this work has provided another example of the great disparity between the first and succeeding members in a homologous series, since the products and conditions of oxidation are very different from those found for glycine. The acids formed from the cyanide are not oxidisable, and from a dibasic amino acid, a cyano-acid has been obtained and identified.

DESIGN OF FIELD EXPERIMENTS

The earlier work of the Statistical Department included the designing of field experiments so that a valid estimate could be made of the magnitude of the errors affecting the results, and at the same time as much as possible of the variation due to soil irregularities

(12) A. G. Norman—" The Biological Decomposition of Lignin." Sci. Progress., 1936, Vol XXX, pp. 442-456.
(13) A. G. Norman—" The Decomposition of Lignin in Plant Materials." Trans. 3rd Internat. Cong. Soil Sci., Oxford, 1935, Vol. III, pp. 105-108.
(14) A. G. Norman—" The Destruction of Oak by the Death-watch Beetle." Biochem. Journ., 1936, Vol. XXX (in press).
(15) M. F. Norman—" The Oxidation of Amino-acids by Hypochlorite, I, Glycine." Biochem.. Journ., 1936, Vol. XXX, pp. 484-496.

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