LIX. THE HEMICELLULOSES. I. THE HEMICELLULOSE OF WHEAT FLOUR.

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SOME years ago, Schulze described the preparation of nitrogen-free residues from leguminous seeds, which yielded on hydrolysis with dilute acids reducing sugars. The precursors of these sugars which were not actually isolated were called by Schulze "hemicelluloses¹."

During the course of some researches on the constituents of the cell-wall substances of plants by Clayson, Norris and Schryver [1921], it was found that, by means of *N* sodium hydroxide solution, substances could be extracted from all the materials investigated, which were soluble in the more concentrated alkaline solutions, but not in very weak alkali; they could be precipitated from the alkaline solutions on addition of acids, and as they yielded reducing sugars on hydrolysis with acids, they were assumed to belong to the class of products termed by Schulze the "hemicelluloses." Most of the products isolated gave on hydrolysis relatively large amounts of pentoses, and all of them gave a blue coloration with iodine.

It was decided to continue investigations with the object of isolating in bulk these so-called "hemicelluloses" and of determining their distribution and later on their function in plant-tissues. The distribution appears to be a very wide one. In some lignified tissues they exist in relatively large quantities ("wood-gums"), and the product isolated many years ago from barley by O'Sullivan and called by him "amylan" also apparently belongs to this class.

The first investigations were carried out on leguminous seeds from which a "hemicellulose" and also "cytopectic acid" [see Clayson, Norris and Schryver, 1921] were isolated and in extending the work to cereals wheat flour was chosen as the chief material for research. By means of the process described in detail below, a product giving a blue coloration with iodine like starch, but differing in many essential properties from this substance, was isolated. It is shown in the second communication [Schryver and Thomas, 1923] that the same product can also be isolated from the commercial starches.

¹ A fuller account of the literature of the hemicelluloses is given in the third paper of this series by Miss O'Dwyer [1923].

EXPERIMENTAL.

Extraction of gliadin. Flour in quantities of 1 kilo. at a time was heated in tin-lined copper vessels, provided with a reflux condenser, with 8 litres of 70 % alcohol. An efficient stirrer, mechanically driven, was attached to the apparatus and the whole mass kept stirred whilst the mixture of flour and alcohol was heated in a water-bath. After about two hours the alcohol was filtered off from the flour on a Buchner funnel, and the flour was returned to the extraction apparatus and treated a second time with 70 % alcohol. By this means nearly all the gliadin was extracted from the flour. This, after the second filtration from the alcohol, was air-dried.

Extraction of the starch. The dried gliadin-free flour was then ground up to a thick paste with water by a motor-driven pestle and mortar and the paste was then thrown into nearly boiling water. About 9 litres of water altogether were used for each kilo. of flour. The mixture of flour and water (which was made in enamelled buckets) was then kept for 1 hour at about 85° by immersing the containing vessel in a bath of boiling water and was stirred from time to time. In this way the starch was gelatinised. The mass was then cooled to 40° and 1 g. of taka-diastrase, dissolved in about 500 cc. of water at 40° was added gradually with constant stirring. Toluene and chloroform were then added and the mixture was kept for about 20 hours in an incubator at 37°. After this period, the mixture had lost its gelatinous consistency, and was filtered through coarse muslin, with hand pressure, to separate the undigested lumps. These were afterwards ground up with water in a motor-driven pestle and mortar, and the paste thus obtained either added to the main bulk of the filtrate or to the second digestion mixture obtained by the method about to be described. The filtrate was then passed two or three times through a Sharples centrifuge, using first a wide, and afterwards a finer nozzle, the centrifuging process being repeated (generally three times) until the liquid was nearly clear. The solid matter was then obtained in the form of a thick paste. This was then ground up with water, and, as before, the paste was thrown into nearly boiling water, 9 litres again being used. The mixture was kept at about 80° for 1 hour and again digested with taka-diastrase. After the second digestion, the mass usually passed through coarse muslin without pressure and contained very few lumps. The solid was again separated from the liquid by the Sharples centrifuge, and gelatinised and redigested with taka-diastrase as before, for a third time. In the third digestion, only about half the bulk of water used in the first digestions was employed. In the earlier experiments the course of digestion was followed out by estimations of sugar. It was found that by far the greater part of the starch was digested on the first treatment with taka-diastrase. Practically no sugar was obtained as the result of the third digestion, but this was always carried out as a precautionary measure. The starch-free product was obtained finally in the form of a thick paste.

Extraction of the glutenin. The glutenin is soluble in 0.1 % sodium hydroxide

solution, which is too weak to extract the hemicellulose. In some experiments, the starch-free product obtained as above was dried in a current of air before the extraction of the glutenin. It was then obtained in the form of a very hard horny mass, from which the glutenin is extracted only with very great difficulty. It is therefore advisable to extract the glutenin from the starch-free paste without drying previously. This is accomplished by repeated extractions with weak alkali, the solid being afterwards separated from the liquid by means of the Sharples centrifuge. The extraction should be continued until the liquid no longer gives the biuret reaction. It is difficult to get out the last traces of nitrogen, but these can be eliminated by methods of purification described below.

Extraction of the hemicellulose. The mass remaining after extraction of the glutenin is then treated with cold 4 % solution of sodium hydroxide. An appreciable amount of yellowish amorphous substance remains undissolved. This contains a small amount of cytopectic acid, which could be extracted from it by 0.5 % ammonium oxalate solution by the method previously described [Clayson, Norris and Schryver, 1921]. The remainder consisted of cellulose, which dissolved completely in zinc-chloride-hydrochloric acid solution. The insoluble mass was separated from the liquid by filtration through a thick pad of paper pulp (made up with 4 % caustic soda solution). The filtrate was slightly opalescent. On acidification with acetic acid, it became at first very turbid, but on standing for a short time a very bulky white precipitate separated, leaving a perfectly clear white supernatant fluid. This was filtered off on to a large filter, thoroughly washed with water, graded strengths of alcohol, and then with ether and finally air-dried. It was thus obtained in the form of a light white powder.

Properties of the hemicellulose. When prepared in the manner just described, the flour hemicellulose dissolves fairly readily in boiling water, giving a perfectly limpid solution, from which the hemicellulose very readily separates again on cooling. The solution can be filtered through paper in a hot water funnel. The hemicellulose on drying at 100° loses about 50 % of its weight, and the apparently dry powder holds large quantities, therefore, of the liquids which have been used in its preparation. After drying at 100° it is obtained in the form of a hard granular powder, or sometimes of a horny mass. When in this condition, it does not dissolve readily in water boiling under atmospheric pressure. It readily dissolves, however, when heated with water in an autoclave to 120°. From this solution it readily separates in a non-crystalline form on cooling. It gives a blue coloration with iodine just as ordinary starch does, but resists digestion with taka-diastrase. It does not reduce Fehling's solution; when boiled with 1 % sulphuric acid it undergoes hydrolysis, yielding glucose (see next paper). This hydrolysis takes place rapidly and is nearly complete after 2 or 3 hours. As usually prepared, it contains small quantities of nitrogen. It can be purified either by allowing it to separate from its filtered solution in hot water, or by means of the copper salt, a method which is described in detail in the following paper. By these methods it can

be obtained quite free from nitrogen and ash, and then shows a rotation of $[\alpha]_D = +150^\circ$. It is in all respects identical with the hemicellulose obtained from starches, which are described in the paper just quoted.

Origin of the hemicellulose. The hemicellulose was first prepared from flours, purchased in shops, of which the origin was quite unknown. Very varying yields were obtained from different flours. It was of interest to ascertain what the origin of this "hemicellulose" was. It was conceivable that it was an intermediate product derived by the digestion of ordinary starch by taka-diastrase. On the other hand, it might be a distinct entity derived either from the starch grains, or from a different part of the plant cell (possibly the cell-wall). To test these points, an estimation of the cellulose was made from two flours of known origin. These were kindly supplied by Dr A. E. Humpharies. They were both obtained from the same mixture of Australian, English and Manitoba wheat. One flour was, however, a "straight-run" flour corresponding to a yield of 73 % of the wheat, whereas the other was more refined and corresponded only to a 23 % yield. It was found that the former gave a yield of 5.4 % hemicellulose (calculated as dried at 100° and protein-free) whereas the latter gave a yield of only 3.6 %. These experiments were each carried out with 1 kilo. of flour. The hemicellulose obtained from each sample (by the methods described above) was not completely purified, but the nitrogen and dry weight were estimated in each. These preliminary experiments on which not too much weight can be put, indicate that the hemicellulose is not derived from the endosperm.

It is possible that the hemicellulose in flour may exert a considerable influence on its value for bread-making and it is proposed to investigate this question in detail.

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SUMMARY.

From wheat flour can be isolated a substance which resists the action of taka-diastrase, and is soluble in hot water, in which it gives a perfectly limpid solution from which it separates readily on cooling in an amorphous form. It is soluble in *N* NaOH, from which solution it can be precipitated by acids. It can be purified either by separation from its solution in hot water, or by means of its copper salt, and when thus obtained, nitrogen- and ash-free, has a rotation in *N*/2 NaOH of $[\alpha]_D^{20} = +150^\circ$.

(*Note.* A fuller description of some of the properties is given in the following paper.)

REFERENCES.

- Clayson, Norris and Schryver (1921). *Biochem. J.* 15, 643.
O'Dwyer (1923). *Biochem. J.* 17, 501.
Schryver and Thomas (1923). *Biochem. J.* 17, 497.