Literature Cited

(1) Bailey, C. H., "Constituents of Wheat and Wheat Products," Reinhold, New York, 1944.
(2) Balls, A. K., Hale, W. S., *Cereal Chem.* **15**, 622–8 (1938).
(3) Balls, A. K., Hale, W. S., Harris, T. H., *Ibid.*, **19**, 279–88 (1942).
(4) Blish, M. J., *Advances in Protein Chem.* **2**, 337–59 (1945).
(5) Bridges, R. G., *J. Sci. Food Agr.* **6**, 261–8 (1955).
(6) Cookson, M. A., Coppock, J. B. M., *Ibid.*, **5**, 8–19 (1954).
(7) Coppock, J. B. M., Cookson, M. A., Laney, D. H., Axford, D. W. E., *Ibid.*, **5**, 19–26 (1954).
(8) Danielsson, C. E., *Biochem. J.* **44**, 387–400 (1949).
(9) Dempster, C. J., Hlynka, I., Anderson, J. S., *Cereal Chem.* **30**, 492–503 (1953).
(10) *Ibid.*, **31**, 240–9 (1954).
(11) Finney, K. F., *Cereal Chem.* **20**, 381–96 (1943).
(12) Gortner, R. A., Hoffman, W. F., Sinclair, W. B., *Ibid.*, **6**, 1–17 (1929).
(13) Hale, W. S., *Ibid.*, **16**, 695–702 (1939).
(14) Hess, K., *Kolloid Z.* **136**, 84–99 (1954).
(15) *Ibid.*, **141**, 61–76 (1955).
(16) Hess, K., *Trans. Am. Assoc. Cereal Chemists* **11**, 153–66 (1953).
(17) Kneen, E., Sandstedt, R. M., *Cereal Chem.* **18**, 237–52 (1941).
(18) Kolthoff, I. M., Stricks, W., Morren, L., *Anal. Chem.* **26**, 366–72 (1954).
(19) Laws, W. D., France, W. G., *Cereal Chem.* **25**, 231–43 (1948).
(20) McCaig, J. D., McCalla, A. G., *Can. J. Research* **19C**, 163–76 (1941).
(21) McCalla, A. G., *Ann. Rev. Biochem.* **18**, 615–38 (1949).
(22) McCalla, A. G., Rose, R. C., *Can. J. Research* **12**, 346–56 (1935).
(23) McElroy, L. W., Clandinin, D. R., Lobay, W., Pethybridge, S. I., *J. Nutrition* **37**, 329–36 (1949).
(24) Mecham, D. K., Mohammad, A., *Cereal Chem.* **32**, 405 (1955).
(25) Mecham, D. K., Weinstein, N. E., *Ibid.*, **29**, 448–55 (1952).
(26) Miller, B. S., Seiffe, J. Y., Shellenberger, J. A., Miller, G. D., *Ibid.*, **27**, 96–106 (1950).
(27) Moran, T., Pace, J., McDermott, E. E., *Nature* **171**, 103–6 (1953).
(28) Olcott, H. S., Mecham, D. K., *Cereal Chem.* **24**, 407–14 (1947).
(29) Osborne, T. B., "Proteins of the Wheat Kernel," Carnegie Inst. Washington, Publ. **84**, (1907).
(30) Pence, J. W., *Cereal Chem.* **30**, 328–33 (1953).
(31) Pence, J. W., Elder, A. H., *Ibid.*, **30**, 275–87 (1953).
(32) Pence, J. W., Elder, A. H., Mecham, D. K., *Ibid.*, **28**, 94–104 (1951).
(33) Pence, J. W., Mecham, D. K., Elder, A. H., Lewis, J. C., Snell, N. S., Olcott, H. S., *Ibid.*, **27**, 335–41 (1950).
(34) Pence, J. W., Olcott, H. S., *Ibid.*, **29**, 292–8 (1952).
(35) Pence, J. W., Weinstein, N. E., Mecham, D. K., *Ibid.*, **31**, 29–37 (1954).
(36) *Ibid.*, pp. 303–11.
(37) Pence, J. W., Weinstein, N. E., Mecham, D. K., *Ibid.*, **31**, 396–406 (1954).
(38) Price, S. A., *Ibid.*, **27**, 73–4 (1950).
(39) Sinclair, A. T., McCalla, A. G., *Can. J. Research* **15C**, 187–203 (1937).
(40) Sullivan, Betty, J. Agr. Food Chem. **2**, 1231–4 (1954).
(41) Udy, D. C., *Cereal Chem.* **30**, 353–66 (1953).
(42) Winteringham, F. P. W., *J. Sci. Food Agr.* **6**, 269–74 (1955).
(43) Winteringham, F. P. W., Harrison, A., Bridges, R. G., Bridges, P. M., *Ibid.*, **6**, 251–61 (1955).
(44) Wiseblatt, L., Wilson, L., McConnell, W. B., *Can. J. Chem.* **33**, 1295–303 (1955).
(45) Wöstmann, B., *Cereal Chem.* **27**, 391–7 (1950).

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CEREAL COMPONENTS

A Review of Carbohydrates of Wheat and Other Cereal Grains

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Water-insoluble, pentosanlike material containing uronic acid, arabinose, and xylose residues is present in the outer portion (bran) of the wheat grain. A similar material is present in corn hulls and oat hulls. Within the wheat kernel are found glucose, fructose, maltose, fructosyl-raffinose, a number of glucofructosans (levosine) found also in barley, at least two pentosans (hemicelluloses) composed of arabinose and xylose, and starch, which is the major carbohydrate component of all cereal grains. Wheat germ contains sucrose, raffinose, and traces of glucose and fructose; exposure of wheat kernels to moisture results in a decrease of the concentration of these sugars. Barley and oat grains contain a polyglucosan in which the glucose units are joined by 1,3- and 1,4- linkages.

Cereal grains form a large and important source of food for both man and animals. It is important, therefore, that the components of the grains, which form the "reactants" of the food technologist, be separated and subjected to careful study, for only in this way are sustained and far-reaching advances likely to be made.

The physical and chemical behavior of wheat flour, for example, has often been related to either the starch or the protein fractions, which together constitute the major portion of the material. However, it has long been realized that a number of carbohydrates other than starch are present and may play important roles in the physicochemical properties of the flours. It is principally to these relatively minor carbohydrate components in wheat and other grains that attention has been directed in recent years.

Cereal grains, indeed all plant seeds,

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are living biological systems and as such contain enzymes which become active when conditions of moisture and temperature are favorable. Some of these enzyme systems are also found in the cereal flours and, as in the parent grains, may cause the chemical modification of some of the carbohydrate and other components, particularly when water is added to the flour. Although such action is important in certain food processes—for example, baking—it hampers chemical investigations. It is imperative, therefore, to inactivate these enzymes before investigations into the natural components of cereal grains or flours are undertaken. This is usually achieved by heating a suspension of the flour in 80 to 85% aqueous ethyl alcohol under reflux for 30 minutes.

Mono- and Oligosaccharides

After the enzymes have been inactivated, the simple sugars may be extracted from the endosperm of cereal grains with 70% aqueous ethyl alcohol and determined quantitatively by chromatographic analysis (*28*, *31*). This involves the separation of the sugars by paper partition chromatography, using a suitable irrigating solvent, such as 1-butanol–ethyl alcohol–water, followed by their elution from the paper with water and subsequent colorimetric determination by the phenol–sulfuric acid method (*21*). In the case of wheat flour the sugars have been found to be glucose (0.01%), fructose (0.02%), sucrose (0.10%), maltose (0.07%), raffinose, and a series of oligosaccharides composed of D-fructose and D-glucose, called glucofructosans (*30*, *37*, *62*). Using 1-butanol–ethyl alcohol–water (4:1:5) as the irrigating solvent in the chromatographic analysis of the above sugar mixture, one glucofructosan moved at the same rate as melibiose, another moved with raffinose, and others of higher molecular weight moved little, if at all, unless the chromatogram was developed for many days (*59*, *62*).

By these chromatographic techniques, the true sucrose content of wheat flour has been shown to be about one tenth of that indicated by the former conventional method of analysis. The discrepancy is principally due to the glucofructosans which, like sucrose, hydrolyze readily with acid. The total glucofructosan content of wheat flour is about 1% (*30*).

By the use of paper chromatographic analysis coupled with the quantitative submicrodetermination of the component sugars by the phenol–sulfuric acid method (*21*) it has been possible not only to determine the nature and amounts of the carbohydrates in a single wheat germ but also to analyze separately the embryonic plant and scutellum sections of the germ for sugars (*20*). It was found (Table I) that the carbohydrates present in wheat germ are principally raffinose and sucrose with a trace of glucose. The amount of these sugars in the whole dormant embryo appears to be dependent upon the highest moisture level to which the grain has been exposed, the total amount of sugars falling as the moisture level rises. A further indication of pregermination metabolic activity caused by moisture is found in the analysis of the scutellum, where the ratio of raffinose to sucrose increases with the moisture level of the grain.

Table I. Soluble Carbohydrate Content of Wheat Germ (*20*)

Sugar	% in Total Embryo	% in Embryonic Plant Section	% in Scutellum Section
Whole Grain Moisture Content, 10.1%			
Total sugar, % dry weight basis	20.1	22.0	18.4
Raffinose, % of total sugars	41.5	45.3	38.1
Sucrose, % of total sugars	58.5	54.7	62.0
Whole Grain Moisture Content, 14.8%			
Total sugar, % dry weight basis	...	17.9	13.6
Raffinose, % of total sugars	...	44.5	51.0
Sucrose, % of total sugars	...	55.5	49.0

Glucofructosans

The glucofructosans of higher molecular weight from wheat (*40*), rye (*16*, *48*, *49*, *52*), and oat (*50*, *53*, *55*) grains appear to be similarly constituted (cf.*54*). These polysaccharides have molecular weights of around 2000 and are very soluble in water. Their aqueous solutions have no viscous characteristics and therefore do not apparently contribute in themselves to the physical characteristics of the flour doughs. However, during the fermentation period in bread making they are rapidly hydrolyzed (*26*) and the fructose so liberated probably forms a principal source of energy supply for the yeast after glucose has been exhausted. It is apparent, therefore, that the amount of glucofructosan, or levosine as it is sometimes called, as well as the simpler sugars mentioned above, will play an important role in the preparation of the dough and will then affect the final product.

The structures of the glucofructosans have been investigated by the classical methylation techniques and recently these studies have been extended and simplified by the use of chromatographic analytical tools. The application of the methylation technique may be illustrated by reference to wheat glucofructosan. This polysaccharide is first treated with methyl sulfate and alkali to convert all the free hydroxyl groups into methoxyl groups. The fully methylated product obtained in this methylation process gives, upon hydrolysis with acid, 2,3,4,6-tetra-*O*-methyl-D-glucose (1 mole), 1,3,4,6 - tetra - *O* - methyl - D - fructose (3 moles), 1,3,4 - tri - *O* - methyl - D fructose (2 moles), and 3,4 - di - *O* - methyl - D - fructose (2 moles) (*40*). The large proportion of the tetra-*O*-methyl derivatives of D - fructose and D - glucose, derived from the nonreducing end residues in the glucofructosan, indicates a highly branched structure for the molecule. The identification of 1,3,4-tri-*O*-methyl-D-fructose shows that in the polysaccharide these residues are linked through positions 2 and 6. The fructose residues which gave rise to 3,4-di-*O*-methyl-D-fructose are clearly joined to other units through position 1 as well as through positions 2 and 6 and hence constitute branch points in the molecule. Since the wheat glucofructosan is nonreducing, one possible structure that will account for these facts is shown in formula I.

Inspection of this formula shows that the G_p1—2 Fru_f portion of the molecule, enclosed by dotted lines, is actually a sucrose residue, whose presence in the glucofructosan is supported by the chromatographic identification of sucrose in the products formed by autohydrolysis (cf. *5*, *6*, *8*) of the glucofructosan. Additional support for this general type of structure for many glucofructosans has been accumulating as a result of studies similar to that described above (*5*, *6*, *9*, *27*, *32*) and also from enzymic investigations (*17*, *18*, *22*, *26*, *43*, *51*). It has been suggested therefore, that the glucofructosans are enzymically synthesized by transfructosidation from a sucrose primer (*9*, *13*, *16*).

In a similar study of a glucofructosan, also obtained from wheat, other investigators (*53*) report the isolation of

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[α-D-Gp1—2 β-D-Fruf6]—2 β-D-Fruf6—2 β-D-Fruf6—2 β-D-Fruf6—2 β-D-Fruf
                          1                        1
                          |                        |
                          2                        2
                       β-D-Fruf                 β-D-Fruf
```

Formula I

3,4,6-tri-*O*-methyl-D-fructose as one of the cleavage fragments of the methylated polysaccharide. This trimethylfructose is different from the 1,3,4-trimethyl derivative found in the authors' investigations. Such a finding indicates an inulin type of structure for the polysaccharide which is radically different from that shown in Formula I, inasmuch as the principal glycosidic linkage would be of the 1,2- rather than the 2,6- type as in I. Such a difference between two wheat glucofructosans, isolated in essentially the same manner, is as yet unexplained. It is conceivable that different glucofructosans are produced by different varieties of wheat, or by the different climatic conditions prevailing in Europe and America, or else there are two different types of glucofructosan in wheat. Some support for the latter view is provided by the suggestion, based on chromatographic evidence, that there are two homologous series of glucofructosans in wheat (*60*).

Table II. Cleavage Products of Methylated Wheat Pentosans

	Molecular Proportions	
Sugar	*Wheat gum pentosan (39)*	*Squeegee pentosan (38)*
2,3,5-Tri-*O*-methyl-L-arabofuranose	13	14
2,3-Di-*O*-methyl-D-xylopyranose	19	24
2-*O*-Methyl-D-xylopyranose	6	7
3-*O*-Methyl-D-xylopyranose	Trace	. .
D-Xylose	4	4

Hemicelluloses or Cereal Gums

Substances known as cereal gums are obtained by extracting the endosperm of cereal grains with water and adding alcohol to the extract. These cereal gums, although largely polysaccharide in nature, also contain a certain amount of protein. They give viscous solutions in water and they may well play an important role in the physical characteristics of the doughs of cereal flours. The polysaccharides of cereal gums are composed of glucosans and araboxylans, the latter belonging to the groups of polysaccharides known as pentosans or hemicelluloses. Constitutional studies on the cereal gum polysaccharides therefore involve a preliminary fractionation step to separate the glucosans from the pentosans. In general little separation of polysaccharide mixtures is achieved by fractional precipitation from aqueous solution with alcohol or acetone because of the highly associative intermolecular hydrogen bonding forces between the two polysaccharides (*23, 39*). Most success in separating the polysaccharides for structural studies is afforded by the fractional precipitation of the acetyl or methyl derivatives, although in the case of the cereal gum from barley flour, separation of the two polysaccharides was achieved by the controlled addition of ammonium sulfate (*47*). This method of separating polysaccharides, used in only a few cases (*57*), appears to be worthy of a much closer study.

The gum from wheat amounts to about 1 to 1.5% of the flour, of which only the hemicellulose component has received any detailed structural study (*39, 44*). This water-soluble pentosan is composed of approximately two parts of D-xylose and one part of L-arabinose. Upon methylation it gives rise to a methyl derivative, the cleavage fragments of which have been shown to be those listed in Table II. It is seen that L-arabofuranose units constitute the end groups of the polysaccharide and that the high proportion of this residue indicates a highly branched structure. When taken into consideration with the results of graded hydrolysis (*44*), it becomes apparent that the general structure of this pentosan consists of a linear framework of 1,4-linked D-xylopyranose units with L-arabofuranose side chain residues attached to certain D-xylose units of the framework principally through position 3 as shown in Formula II. There is also the possibility that branching may occur through position 2 of a small number of the D-xylopyranose units, for this would explain the isolation of 3-*O*-methyl-D-xylose.

When wheat flour, freed from gluten, is suspended in water and centrifuged, it separates into a tightly packed lower layer of starch, above which is a mucilaginous material. This mucilaginous component has been called the "amylodextrin" or "squeegee" or "tailings" of wheat flour. Treatment of the mucilaginous material (*15*) with pancreatin gives a water-insoluble carbohydrate substance (*56*) which consists of a glucosan and a pentosan. Upon fractionation, by extraction of the acetate of the polysaccharide complex with acetone, the acetone-insoluble portion, after deacetylation, gives a relatively pure hemicellulose which is soluble in water (*38*). As will be seen from the cleavage products of the methylated squeegee pentosan summarized in Table II, the building units of this pentosan are similar to those of the hemicellulose isolated from wheat gum. Although the general structural features of the wheat flour water-soluble pentosan and the squeegee starch pentosan are similar, the difference in their physical properties indicates that the latter possesses less linear character than the former. One of the striking features of the squeegee pentosan is the highly viscous nature of its methyl derivative, which would indicate that the viscosity of the material is due not only to association caused by hydrogen bonding but also to molecular shape and physical entanglement of the molecules. It is possible that these wheat gums may play an active part in the rheology of flour doughs. Apparently these pentosans or hemicelluloses are not changed by the baking and staling processes of bread, as was indicated by a study of the pentosan from stale bread (*11, 12, 23*).

The so-called "soluble starch" from bread crumb contains 10 to 50% of a pentosan, depending upon the freshness of the bread (*23*). The proportion of pentosan to glucosan increases as staling progresses, thus indicating a retrogradation of glucose-containing polymers (amylose, amylopectin).

Barley gum (*34, 35, 42, 45–47*), obtained by an aqueous extraction of barley flour, and to which may be ascribed some of the malting and resulting wort qualities, also contains a pentosan which appears to be similar to that found in wheat (*25*). Both barley and wheat pentosans have high negative rotations $[\alpha]_D$ $-100°$ to $-110°$, and their methyl ethers have still higher negative rotations $[\alpha]_D$ $-160°$ to $-170°$; both polysaccharides are highly branched and both have a framework of D-xylopyranose residues (see formula II). The main structural features of the barley gum pentosan follow from the fact that hydrolysis of the methylated product furnishes 2,3,5-tri-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose, and D-xylose.

L-Ara$_f$1—[4 β-D-Xyl$_p$1]$_x$—4 β-D-Xyl$_p$1—[4 β-D-Xyl$_p$1]$_y$—
(on the central Xyl, position 3 ← 1 L-Ara$_f$)

II

Also in barley gum is a poly-β-glucosan which gives viscous solutions in water and which undergoes enzymatic breakdown during malting (*47*). Structurally it is composed of β-glucopyranose residues, which from methylation studies are shown to be linked through positions 1 and 4 and through 1 and 3, the two types of linkage being present to about the same extent (*7*). The ratio of 1,4- to 1,3- linkages in barley glucosan has also been determined by periodate oxidation (*24*), by making use of the fact that the 1,3-linked residues will resist oxidation while each 1,4-linked glucose unit, possessing two adjacent hydroxyl groups, will consume one mole of periodate. Thus, if, as in barley β-glucosan, the two types of linkage are present in equal amounts, then one mole of periodate will be consumed by one out of every two glucose residues. The 1,4- and 1,3- linkages in the polyglucosan appear to alternate as

$$—3\ \beta\text{-D-G}_p1—[4\ \beta\text{-D-G}_p1—3\ \beta\text{-D-G}_p1]_n—4\ \beta\text{-D-G}_p1—$$

III

in Formula III, since the polyaldehyde, produced by periodate oxidation, gives rise to glucose phenylosazone when treated with phenylhydrazine (*24*). The presence of glucose residues joined by consecutive 1,3-glycosidic bonds would be revealed by the formation of osazones of one or more glucose oligosaccharides and not simply the osazone of the monosaccharide, glucose.

In the case of a similar glucosan isolated from oats, called oat lichenin (*41*), periodate oxidation studies have indicated the proportion of 1,4- to 1,3-linkages to be about 3 to 1 (*2*); a similar polyglucosan with a still higher proportion of 1,4-linkages has been found in Iceland moss (*14*, *36*).

Some support for the presence of 1,3-linked polysaccharides in other cereal grains, if only in small amount, is forthcoming from the observation that an enzyme is present in wheat and many other seeds which is capable of hydrolyzing lichenin and the 1,3-β-linked polysaccharide, laminarin (*19*, *29*, *33*, *48*). The gradual accumulation of such evidence as this lends some indirect support to the view (*1*), based on periodate oxidation studies, that a few 1,3- linkages may be present in starch.

Accompanying the poly-β-glucosan in barley gum is an α-glucosan which has been shown by methylation studies to be similar to the amylopectin fraction of starch (*25*). It differs only in the degree of branching, barley α-glucosan being much more highly branched, with each repeating unit containing on the average eight glucopyranose units (see Formula IV) as compared with an average repeat-

$$\alpha\text{-D-G}_p1—[4\ \alpha\text{-D-G}_p1]_m—4\ \overset{\overset{|}{6}}{\alpha\text{-D-G}_p1}—[4\ \alpha\text{-D-G}_p1]_n—$$

IV

ing unit of about 20 glucopyranose residues found in amylopectin. This has been deduced from the fact that hydrolysis of the methylated polysaccharide yields 2,3,4,6-tetra-*O*-, 2,3,6-tri-*O*-, and 2,3-di-*O*-methyl-D-glucose.

The same types of hexosans as the α- and β-glucosans of barley may well be present in all cereal grains.

The outer coating or pericarp of cereal grains contains a high proportion of pentosan material which appears to be more complex than those found in the endosperm. In the case of wheat bran the pentosan is composed of L-arabinose, D-xylose, D-glucuronic acid, and 4-*O* methyl-D-glucuronic acid. Structural studies by the classical methylation technique showed that the methylated pentosan gave rise upon hydrolysis to eight neutral methylated sugars (see Table III) (*3*).

It is apparent from these results and relative hydrolysis rates of the arabinose residues that some of these are part of the central portion of the hemicellulose molecule. The isolation of 2,3,5-tri-*O*-methyl-L-arabinose and 2,3,4-tri-*O*-methyl-D-xylose shows that the end groups in the polysaccharide are composed of both L-arabofuranose and D-xylopyranose units. Both of these structural features are in contrast to those of the wheat endosperm hemicellulose, which contains L-arabofuranose only as terminal units. A further difference between the two hemicelluloses is the presence of acidic groups in that from the bran. By partial acid hydrolysis of the bran hemicellulose the acidic components have been isolated as aldobiouronic acids, in which the acidic moiety has been shown to be linked through its reducing group to a D-xylose unit at position 2 (*4*). It is probable that these acidic residues also form end groups in the hemicellulose, and as they are probably present as salts, the explanation for the higher ash content of the bran as compared to the endosperm becomes apparent. A similar hemicellulose, $[\alpha]^{22}_D -96°$ (1*N* sodium hydroxide), may be extracted from oat hulls with dilute alkali (*58*).

Table III. Neutral Cleavage Fragments of Methylated Wheat Bran Hemicellulose (3)

Component	*Proportions*
L-Arabinose	
2,3,5-Tri-*O*-methyl-	6
2,5-Di-*O*-methyl-	7
3-*O*-Methyl-	(?) 3
5-*O*-Methyl-	3
D-Xylose	
2,3,4-Tri-*O*-methyl-	5
2,3-Di-*O*-methyl-	4
2-*O*-Methyl-	4

Table IV. Carbohydrate Components of Wheat

Glucose	Glucofructosans
Fructose	Araboxylans
Sucrose	Amylopectin
Maltose	Amylose
Raffinose	Other glucosans
Fructosyl-raffinose	

A similar hemicellulose extracted from the pericarp of corn with dilute alkali is composed of arabinose, xylose, galactose, and glucuronic acid (*40*, *63*). This polysaccharide forms gummy solutions in water, which, though lower in viscosity than those of similar concentration made from gum Karaya and tragacanth, are considerably higher than those of gum arabic (*63*). Like wheat bran hemicellulose, the corn hull polysaccharide has been shown by methylation studies (*40*) to have end groups of arabinose, xylose, and glucuronic acid. The L-arabinose and D-xylose have been obtained as the 2,3,5-tri- and the 2,3,4-tri-*O*-methyl derivatives, respectively. The nonreducing end groups of D-glucuronic acid were obtained as a partially methylated aldobiouronic acid from the methylated polysaccharide and characterized, after reduction, with lithium aluminum hydride as crystalline 2-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyl)-3-*O*-methyl-D-xylose. The characterization of the latter also proved that the terminal units of D-glucuronic acid were joined directly to the main structural xylan framework of the polysaccharide. This corn hull hemicellulose differs from wheat bran hemicellulose in also having the galactose residues as nonreducing end groups. The framework of corn pericarp hemicellulose, like that from wheat bran, is composed principally of D-xylopyranose residues linked through positions 1 and 4 and from which are subtended multiunit side chains containing arabinose residues. This follows from the characterization of 2,3-di and 2-*O*-methyl-D-xylose among the cleavage products of the methylated polysaccharide. Similar results have been obtained by other investigators, who have carried out methylation (*10*) and degradation (*59*) studies on a corn "fiber" hemicellulose which is very similar if not identical with the substance referred to above as corn hull hemicellulose.

Conclusion

In order to illustrate the diversity of the carbohydrate components in cereal grains, those found thus far in wheat are listed in Table IV. There are the simple sugars and oligosaccharides, including the recently identified fructosyl-raffinose (*61*); the glucofructosans, which may be considered as a spectrum of oligosaccharides with sucrose as the simplest member; the highly branched araboxylans, which represent what might be termed the cellular cement of most plants; the branched and linear components of wheat starch, which, together, represent by far the principal carbohydrates in wheat; and, finally, the other glucosans about which little is known as yet. No mention is made here of the glycoproteins, which undoubtedly play a major role in cereals and other plant products.

It is the authors' belief, however, that the full chemical and food technological utilization of the vast store of carbohydrates, proteins, lipides, and their complexes present in wheat and other cereal grains, which in contrast to other natural sources of organic chemicals like oil is reproducible annually, will not be realized until the fundamental approaches to the separation, identification, and structure determination of their components have been fully exploited.

Literature Cited

(1) Abdel-Akher, M., Hamilton, J. K., Montgomery, R., Smith, F., *J. Am. Chem. Soc.* **74**, 4970 (1952).
(2) Acker, L., Diemair, W., Samhammer, E., *Z. Lebensm.i Untersuch. u- Forsch.* **100**, 180 (1955).
(3) Adams, G. A., *Can. J. Chem.* **33**, 56 (1955).
(4) Adams, G. A., Bishop, C. T., Abstracts of Papers, ACS, 128th meeting, p. 7E, 1955.
(5) Arni, P. C., Percival, E. G. V., *J. Chem. Soc.* **1951**, 1822.
(6) Aspinall, G. O., Hirst, E. L., Percival, E. G. V., Telfer, R. G. J., *Ibid.*, **1953**, 337.
(7) Aspinall, G. O., Telfer, R. G. J., *Ibid.*, **1954**, 3519.
(8) *Ibid.*, **1955**, 1106.
(9) Bell, D. J., Palmer, Anne, *Ibid.*, **1952**, 3763.
(10) BeMiller, J. N., Whistler, R. L., Abstracts of Papers, ACS, 128th meeting, p. 4D, 1955.
(11) Bice, C. W., Ph.D. thesis, University of Minnesota, 1950.
(12) Bice, C. W., Geddes, W. F., *Cereal Chem.* **26**, 440 (1949).
(13) Boggs, L. A., Cuendet, L. S., Dubois, M., Smith, F., *Anal. Chem.* **24**, 1148 (1952).
(14) Boissonnas, R. A., *Helv. Chim. Acta* **30**, 1703 (1947).
(15) Clendenning, K. A., Wright, D. E., *Can. J. Research* **28F**, 390 (1950).
(16) Cuendet, L. S., Ph.D. thesis, University of Minnesota, 1950.
(17) Dedonder, R., *Bull. soc. chim. biol.* **34**, 144, 157, 171 (1952).
(18) Dedonder, R., *Compt. rend.* **232**, 1442 (1951).
(19) Dillon, T., O'Colla, P., *Nature* **166**, 67 (1950).
(20) Dubois, M., M.S. thesis, University of Minnesota, 1951.
(21) Dubois, M., Gilles, K., Hamilton, J. K., Rebers, P. A., Smith, F., *Nature* **168**, 167 (1951).
(22) Edelman, J., Bacon, J. S. D., *Biochem. J.* **49**, 446, 529 (1951).
(23) Gilles, K. A., Ph.D. thesis, University of Minnesota, 1952.
(24) Gilles, K. A., Huffman, G. W., Meredith, W. O. S., Smith, F., unpublished work.
(25) Gilles, K. A., Meredith, W. O. S., Smith, F., *Cereal Chem.* **29**, 314 (1952).
(26) Grandchamp-Chandun, Andrée de, *Compt. rend.* **231**, 1082 (1950).
(27) Hirst, E. L., McGilvray, D. I., Percival, E. G. V., *J. Chem. Soc.* **1950**, 1297.
(28) Hough, L., "Methods of Biochemical Analysis," D. Glick, Ed. Vol. **1**, pp. 205–42, Interscience Publishers, New York, 1954.
(29) Karrer, P., Staub, M., *Helv. Chem. Acta* **7**, 518 (1924).
(30) Koch, R. B., Geddes, W. F., Smith, F., *Cereal Chem.* **28**, 424 (1951).
(31) Kowkabany, G. N., *Advances in Carbohydrate Chemistry* **9**, 304 (1954).
(32) Laidlaw, R. A., Reid, S. G., *J. Chem. Soc.* **1951**, 1830.
(33) Lüdtke, M., *Biochem. Z.* **323**, 428 (1953).
(34) Meredith, W. O. S., Anderson, J. A., *Cereal Chem.* **32**, 183 (1955).
(35) Meredith, W. O. S., Bass, E. J., Anderson, J. A., *Ibid.*, **28**, 177 (1951).
(36) Meyer, K. H., Gurtler, P., *Helv. Chim. Acta* **30**, 751 (1947).
(37) Montgomery, R., Smith, F., *Cereal Chem.* **31**, 490 (1954).
(38) Montgomery, R., Smith, F., *J. Am. Chem. Soc.* **77**, 2834 (1955).
(39) *Ibid.* p. 3325.
(40) Montgomery, R., Smith, F., unpublished work.
(41) Morris, D. L., *J. Biol. Chem.* **142**, 881 (1942).
(42) O'Sullivan, C., *J. Chem. Soc.* **41**, 24 (1882).
(43) Palmer, Anne, *Biochem. J.* **48**, 389 (1951).
(44) Perlin, A. S., *Cereal Chem.* **28**, 382 (1951).
(45) Preece, I. A., *European Brewery Convention, Proc. Congr. Brighton* **1951**, 213.
(46) Preece, I. A., Ashworth, A. S., Hunter, A. D., *J. Inst. Brewing* **56**, 33 (1950).
(47) Preece, I. A., MacKenzie, K. G., *Ibid.*, **58**, 353 (1952).
(48) Pringsheim, H., Seifert, K., *Z. physiol. Chem.* **128**, 384 (1923).
(49) Schlubach, H. H., Bandmann, C., *Ann.* **540**, 285 (1939).
(50) Schlubach, H. H., Hanschildt, P., *Ibid.*, **578**, 201 (1952).
(51) Schlubach, H. H., Holzer, K., *Ibid.*, **578**, 213 (1952).
(52) Schlubach, H. H., Koenig, K., *Ibid.*, **514**, 182 (1934).
(53) Schlubach, H. H., Müller, H., *Ibid.*, **572**, 106 (1951).
(54) *Ibid.*, **578**, 194 (1952).
(55) Schlubach, H. H., Ratkje, E., *Ibid.*, **561**, 180 (1948).
(56) Simpson, F. J., *Can. J. Microbiol.* **1**, 131, (1954).
(57) Smith, D. B., Cook, W. H., Neal, J. L., *Arch. Biochem. and Biophys.* **53**, 192 (1954).
(58) Smith, F., unpublished work.
(59) Whistler, R. L., Corbett, W. M., Abstracts of Papers, ACS, 128th Meeting, p. 7E, 1955.
(60) White, L. M., Secor, G. E., *Arch. Biochem. Biophys.* **43**, 60 (1953).
(61) *Ibid.*, **44**, 244 (1953).
(62) Williams, K. T., Bevenue, A., *Cereal Chem.* **28**, 416 (1951).
(63) Wolf, M. J., MacMasters, M. M., Cannon, J. A., Rosewall, E. C., Rist, C. E., *Cereal Chem.* **30**, 451 (1953).

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MILK ANALYSIS

Direct Microdetermination of Calcium in Milk

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A RAPID MICROMETHOD for the determination of calcium was required for studies on ionic equilibria in milk. The method had to be suitable for the analysis of small aliquots of synthetic mixtures resembling milk serum but containing as little as 10 γ of calcium per ml. Gravimetric, colorimetric, and titrimetric methods (*1*) were unsatisfactory because they were time-consuming or lacked sensitivity with the limited aliquots of test solution available.

The method of Saifer and Clark (*2*), which estimates from 40 to 280 γ of calcium in water, was studied in detail with the aim of adapting it to milk and milk serum. A successful modified method, with increased sensitivity, measures from