Pentosans Associated with Gluten[1]

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ABSTRACT

A procedure was established for the isolation, purification, and fractionation of the pentosans which remain associated with the gluten fraction of wheat flour. Preliminary investigations on a large number of glutens extracted from different hard red spring wheat-flour varieties indicated that all of the glutens contained pentosan material. Included in the study were glutens extracted from durum semolina and soft wheat flour. Initially, various methods were attempted for extracting pentosans from the gluten, with the following approach finally adopted. The procedure involved sodium hydroxide extraction of the gluten followed by neutralization with acetic acid and subsequent centrifugation. The extract was then subjected to heating followed by centrifugation and dialysis of the supernatant. The freeze-dried crude pentosan preparation was subjected to pancreatin treatment and ethanol precipitation for further purification. DEAE-cellulose chromatography was used to fractionate the pentosans into five fractions. Fraction I was essentially an arabinoxylan with only small amounts of protein. The component sugars in the fractions were examined by paper and gas-liquid chromatography. Intrinsic viscosity and optical rotation were used to further characterize the different fractions. The results appear to indicate that the pentosans associated with gluten are similar to the pentosans extracted from flour by water. Pentosans extracted from durum glutens contained higher amounts of pentosans and appeared to have a greater degree of branching as well as a higher intrinsic viscosity.

Several investigators have examined the chemical and physical properties of water-soluble pentosans from wheat flour. Included among a few of the latest studies are those of several workers (1,2,3). Pentosans associated with the sludge or tailings fraction of wheat flour have also been studied by several investigators, among which two of the most recent include the studies of Cole (4) and of Medcalf and Gilles (5).

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It is generally agreed that wheat endosperm pentosans are composed of a main chain of D-xylopyranosyl units linked beta-1,4 with L-arabinofuranosyl branches occurring either at the 2- or 3-positions of the xylose units. Also, pentosans of wheat flour are normally quite closely associated with proteinaceous material and require considerable purification before obtaining an essentially pure arabinoxylan.

To date, no intense effort has been made to examine the pentosans which remain associated with the gluten fraction of wheat flour. Although the effect of water-soluble pentosans in baking has been investigated by numerous workers (6–12), their complete role has not as yet been fully elucidated. It may be that their importance is not as a single entity but rather as a carbohydrate-protein complex. It may also be possible that the pentosans associated with gluten play a role in baking quality.

This paper reports on a procedure utilized for the extraction of pentosans from glutens of different sources as well as an investigation of the properties of the isolated pentosans.

MATERIALS AND METHODS

Wheat Samples

The flour and semolina samples utilized for the investigation of pentosans associated with gluten were milled from pure varieties of hard red spring (HRS), durum, and soft wheats on a Buhler mill.

Gluten Isolation

Gluten was isolated from the flour or semolina by the dough-kneading procedure. A dough was made from 500-g. portions of flour or semolina and 300 ml. of distilled water. Starch and soluble material were removed by hand-washing of the dough ball. The freeze-dried gluten was ground on a Wiley mill to pass a No. 30 sieve.

Determination of Pentosan Content in Gluten

The amount of pentosans in the different glutens was determined by the volumetric bromine method according to the AACC procedure (13). A sample size of 0.50 g. of gluten was used for the determination, while in a number of cases, 1.0 g. was used to check the results.

Isolation of Pentosans Associated with Gluten

The general procedure used for the isolation, purification, and fractionation of the pentosans associated with the gluten is outlined in Fig. 1. Considerable preliminary work was performed prior to adopting the above-mentioned procedure. Early studies involved variations in the solvent-to-solid ratio used in the extraction, variation in sodium hydroxide (NaOH) concentration, ammonium sulfate precipitation, and various enzyme treatments.

The procedure finally adopted was as follows: Gluten (150 g.) was extracted under nitrogen with 1,250 ml. of 0.2N NaOH with stirring for 30 min. The supernatant was collected after centrifugation at 10,000 × g for 15 min. The residue was re-slurried with 750 ml. of 0.2N NaOH and stirred under nitrogen for 30 min. After centrifugation, the supernatants were combined, neutralized with glacial acetic acid, and centrifuged until clear. Next, the extract was heated to

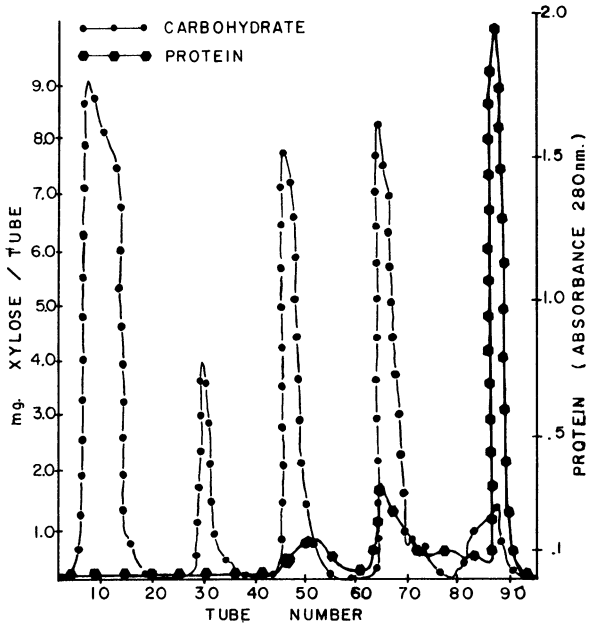

Fig. 1. Schematic diagram for isolation, purification, and fractionation of pentosans associated with gluten.

92°C., held at that temperature for 4 min., cooled, and centrifuged. The supernatant was dialyzed for 2 days against distilled water, recentrifuged until clear, shell-frozen, and freeze-dried. This represented the crude pentosan preparation which was subjected to pancreatin treatment by the procedure of Simpson (14) with minor modifications. A 2.5% solution of the pentosans was prepared and the pH adjusted to 7.2 to 7.4. A 2% centrifuged solution of pancreatin in 0.1% sodium chloride was added to the pentosan solution and the mixture digested at room temperature for 5 hr. The pH was adjusted to 8.0 and the sample stored overnight in the refrigerator. The following day the solution was heated to 92°C., held at that temperature for 5 min., cooled, and centrifuged. Ethanol was added in a 1:4 ratio and the solution allowed to precipitate in the cold overnight. The precipitated pentosan preparation was collected by centrifugation, dissolved in water, shell-frozen, and freeze-dried.

DEAE-Cellulose Chromatography

Two sizes of columns and two types of DEAE-cellulose were employed. One column was 2 X 45 cm.; the other was a smaller column measuring 2 X 20 cm. The two types of DEAE-cellulose employed were DE 11 (1.0 meq. per g.) and DE 23 (1.0 meq. per g.). DE 11 was used in earlier experiments.

The smaller column packed with DE 23 was used to fractionate the pancreatin-treated gluten pentosans extracted from a number of HRS wheat flour glutens. With this column, only two fractions were collected, using distilled water and 0.4N NaOH as eluants, respectively. The purpose of the experiment was primarily to examine fraction I and ascertain the yield of this fraction as well as the

ratio of arabinose to xylose to see if any differences were observable in the glutens from different varieties.

Fractionation on the larger column included early experiments in which the crude pentosans, identified as pentosan material prior to pancreatin treatment, were fractionated with DE 11 using three eluants: distilled water, 0.100M sodium borate, and 0.3N NaOH.

When the procedure shown in Fig. 1 was employed in which the pentosans were subjected to pancreatin treatment, five fractions were collected from the DEAE-cellulose column. Both types of DEAE-cellulose were utilized and the differences observed.

With the larger column, the sample (200 to 300 mg.) was dissolved in 10 ml. distilled water and applied to the top of the column. After the sample was allowed to penetrate into the DEAE-cellulose, elution was accomplished with the following eluants: distilled water, 0.0025M $Na_2B_4O_7$, 0.025M $Na_2B_4O_7$, 0.125M $Na_2B_4O_7$, and 0.4 N NaOH, respectively. Each tube collected was measured at 280 or 260 nm. for protein analysis. The carbohydrate content in each tube was estimated by the orcinol procedure (15). Tubes corresponding to each carbohydrate peak were combined, dialyzed to remove inorganic ions, and freeze-dried. The protein content of each fraction was estimated by the Folin-Ciocalteu method as modified by Lowry et al. (16).

Paper Chromatography

Paper chromatography was used to examine qualitatively the hydrolyzed pentosan fractions as well as the unfractionated material. Whatman No. 1 paper was used with ethyl acetate:pyridine:water (10:4:3 v./v.) as solvent. Sugars were visualized with silver nitrate spray reagent (17).

Ratio of Component Sugars

The ratio of component sugars in the various DEAE-cellulose pentosan fractions was determined as described by Medcalf et al. (3) with slight modifications. After hydrolysis with sulfuric acid, reduction with sodium borohydride, and acetylation with acetic anhydride and pyridine, the sample was concentrated under reduced pressure, taken up in chloroform, and concentrated again. This process with chloroform was repeated three times. The syrupy residue then was treated with water according to Crowell and Burnett (18) and evaporated to dryness. The resulting acetates were dissolved in ethylene dichloride and injected into the gas chromatograph.

Gas chromatography was performed with a Barber Coleman Series 5000 chromatograph equipped with a flame ionization detector. Separation of the alditol acetates was achieved with an 8-ft. glass column packed with 3% ECNSS-M (Applied Science Laboratories, Inc.) on 100- to 120-mesh Gas Chrom Q. Relative proportions of the various sugars present were calculated from peak areas determined by triangulation.

Optical Rotation and Intrinsic Viscosity

Specific optical rotations of the various pentosan fractions were determined in 0.5N NaOH solution with a Galileo polarimeter.

Intrinsic viscosities were determined on the same fractions in 0.5N NaOH solution at 25°C. with an Ubbelohde viscometer.

RESULTS AND DISCUSSION

The normality and amount of NaOH solution used to extract the pentosans from gluten affected the amount of material recovered. In general, as the NaOH solution was increased from 0.05N to 0.40N, the amount and protein content of the material extracted increased at the same time. Also, as the amount of 0.2N NaOH solution used for the extractions was increased, the total amount of material extracted was greater.

Preliminary studies on the extraction of pentosans from gluten were conducted on a series of glutens. Two sets of experiments were performed. In both experiments, a number of glutens were extracted with 0.2N NaOH solution. However, the amount of NaOH solution used for the extractions varied. In this particular experiment, the pentosans were not subjected to pancreatin treatment.

These pentosans were fractionated on a DEAE-cellulose column, utilizing DE II, into three fractions, using the eluants given in the Methods section. It became evident that further purification of the crude pentosans was required before fractionation on DEAE-cellulose could be accomplished and the fractions examined further. The primary conclusion deduced from the preliminary study was that durum glutens appeared to contain higher amounts of pentosan material.

Treatment of the crude pentosans with crystalline alpha-amylase resulted in three component sugars being present: arabinose, xylose, and galactose. Glucose was not observable by paper chromatography.

The yield of pentosans recovered utilizing the scheme shown in Fig. 1 (which includes the pancreatin treatment step) for several glutens extracted from different sources is shown in Table I. As can be observed, there does not appear to be any great difference in recovery between the different gluten sources. However, when

TABLE I. GLUTEN PENTOSAN DATA

Gluten Source	Pentosan Yield after Pancreatin Treatment %	Pancreatin-Treated Pentosan Protein %	Ratio of Arabinose:Xylose in F_1
HRS			
Neepawa	0.32	39.9	1:1.60
Chris[a]	0.32	48.1	1:1.61
Chris[a]	0.34	41.2	1:1.55
1812	0.32	23.1	1:1.70
RR 68	0.38	43.4	1:1.60
Justin	0.30	42.8	1:1.60
Durum			
Wells	0.35	21.6	1:1.38
Mindum	0.27	23.6	1:1.40
Leeds	0.34	18.1	1:1.41

[a]The two Chris varieties were grown at different locations.

TABLE II. PROTEIN AND PENTOSAN CONTENT OF DIFFERENT GLUTENS

Gluten Source	Protein %	Pentosans %	Gluten Source	Protein %	Pentosans %
HRS			Durum		
Chris[a]	76.7	1.86	Durum composite	66.5	2.68
1812	76.7	1.76	Wells	73.9	2.72
Semidwarf	80.3	1.17	Leeds	80.0	2.20
Chris[a]	78.4	1.78	Mindum	80.5	2.03
RR 68	78.1	1.95			

[a]The two Chris varieties were grown at different locations.

the protein content of the pancreatin-treated pentosans is examined, those from HRS wheat-flour glutens in general were considerably higher than those from durum wheat glutens. This would indicate that more pentosan material is associated with the durum glutens than with the HRS glutens. These observations were confirmed when the pentosan content itself was measured in a number of different glutens. The results shown in Table II also list the protein contents of these glutens. Again, the data presented in Table II support the observation that the durum glutens contain higher amounts of pentosan material. The ratio of component sugars for fraction I of the pancreatin-treated pentosans fractionated on DEAE-cellulose are also given in Table I for several HRS wheat-flour glutens and durum glutens. No appreciable difference was observed in the ratio of arabinose to xylose among the pentosans extracted from HRS wheat-flour glutens or durum glutens. A difference in ratio was observed, however, between pentosans extracted from the HRS and durum glutens. The pentosans associated with the durum glutens appear to have a higher degree of branching than those associated with the HRS glutens. This result has been reported previously with water-soluble pentosans (3).

The average baker's patent flour contains 2 to 3% total pentosans. The yield of pentosans recovered from the different glutens after pancreatin treatment as shown in Table I indicates that only a small percentage of the total flour pentosans is associated with gluten. Also, pentosans extracted from the different glutens were not pure pentosans but contained considerable amounts of protein.

Fractionation of the pancreatin-treated pentosans on a DEAE-cellulose column produced five fractions when distilled water, 0.0025M $Na_2B_4O_7$, 0.025M $Na_2B_4O_7$, 0.125M $Na_2B_4O_7$, and 0.4N NaOH were used as column eluants. The type of DEAE-cellulose used for the fractionation did have an effect on the fractions obtained. The main difference observed when DE II was used compared to DE 23 was in fractions III and IV. With DE II the component sugars found in fraction III were arabinose and xylose in a ratio of almost 1:1 with trace amounts of galactose. Fraction IV, however, was primarily an arabinogalactan with small amounts of xylose. When DE 23 was used which had the same nominal capacity as DE II but which had the short fibers removed, considerable galactose was found in both fractions III and IV. Only small amounts of xylose were present in these two fractions.

Figure 2 shows a representative column fractionation using the DE 23 of a durum gluten pentosan. The orcinol procedure was used to estimate carbohydrate

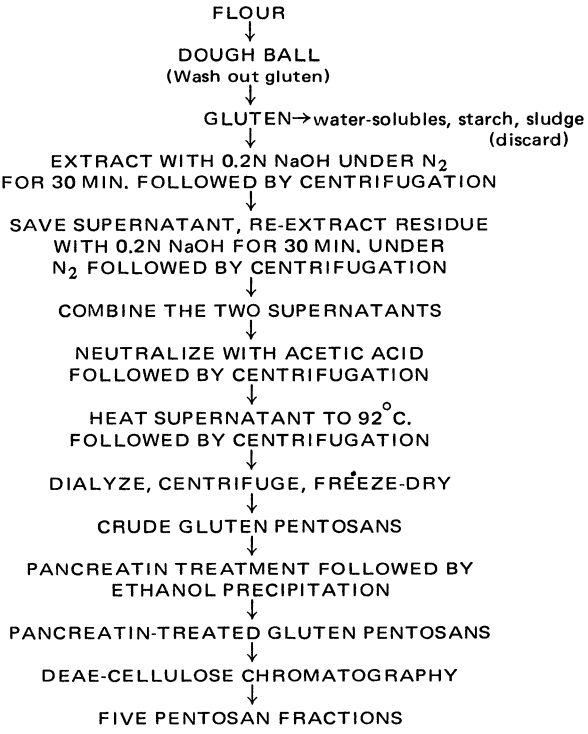

Fig. 2. Column fractionation of pentosans extracted from durum gluten on DEAE-cellulose 23.

content in the tubes, and the protein curve was determined by measurement of the tubes at 280 nm. Fractions I and II did not show any major protein peak, although fraction II, when measured for protein content, did, in the majority of cases, contain substantial amounts of protein. The major protein-containing peak appeared in Fraction V. Figure 3 shows a representative paper chromatogram of the unfractionated pancreatin-treated gluten pentosans and of the five DEAE-cellulose pentosan fractions after hydrolysis in 1N sulfuric acid. No qualitative differences could be observed between the HRS and durum pentosan fractions. Fractions I and II contained arabinose and xylose as component sugars. Fractions III and IV contained primarily arabinose and galactose as component sugars with small amounts of xylose. Fraction V contained arabinose and xylose as component sugars and also glucose with trace amounts of galactose. The glucose observed in fraction V, however, was believed to have come from the DEAE-cellulose during elution with 0.4N NaOH since the unfractionated material did not contain glucose which, if it were present, was present in very, very trace amounts.

Tables III and IV show the yield, protein content, ratio of component sugars, optical rotation, and intrinsic viscosity for three durum gluten pentosans and three HRS gluten pentosans after pancreatin treatment and fractionation on DEAE-cellulose 23.

The yield of fraction I of the durum samples was generally higher than that of

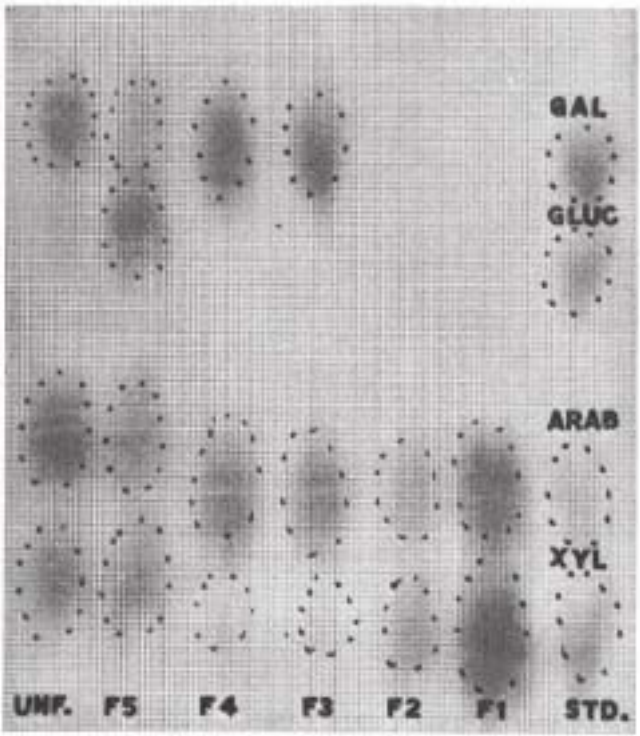

Fig. 3. Paper chromatogram showing sugars present in the hydrolyzed unfractionated gluten pentosans and in the hydrolyzed DEAE-cellulose pentosan fractions.

the corresponding fraction from HRS samples. This result would be in keeping with the previously mentioned observation that the durum glutens contain higher amounts of pentosan than the HRS glutens. The yield of fraction II was very low in all cases. This low yield differed from that reported for this fraction from water-soluble pentosans obtained through the same technique (3). The second major difference regarding yield was that fraction IV, in all cases except one, had the highest yield of the five fractions collected, and was considerably higher in yield than the same fraction for the water-soluble pentosans. Likewise, fraction III of the gluten pentosans was higher in yield than fraction III of the water-soluble pentosans (3). The ratio of component sugars arabinose and xylose for fraction I of the durum gluten pentosans was lower than the ratio of the same fraction for the HRS gluten pentosans. This would indicate a greater degree of branching for pentosans associated with the durum glutens. The ratio of component sugars for the unfractionated gluten pentosans, as well as for fractions III and IV for both the HRS and durum samples, would indicate that the amount of galactose present is higher in these pentosans than that associated with the water-soluble pentosans (3). The ratio of component sugars for fraction V is reported only for arabinose and xylose since it is believed that the glucose observed in the paper chromatogram, as mentioned previously, was derived from the DEAE-cellulose. The highest negative optical rotation was observed in fraction I, which is indicative of beta-type linkage.

TABLE III. DEAE-CELLULOSE PENTOSAN FRACTIONS[a]

Fraction	Yield %	Protein %	Ratio Arabinose: Xylose:Galactose	$[\alpha]_D^{25}$	[n]
Durum (Wells)					
Unfractionated	...	21.6	1:0.71:0.52	...	...
I	25.0	1.4	1:1.38:...	−122°	6.0
II	9.9	6.0	1:1.22:...	−85°	4.3
III	21.5	17.6	1:0.43:0.38	−100°	1.7
IV	30.8	23.7	1:0.26:0.62	−51°	1.0
V	12.8	19.9	1:0.90:...	−51°	2.3
Durum (Leeds)					
Unfractionated	...	18.1	1:0.74:0.48	...	...
I	40.0	2.5	1:1.41:...	−132°	5.1
II	trace	...	...	...	...
III	17.0	11.9	1:0.34:0.60	−96°	1.6
IV	37.7	17.5	1:0.22:0.59	−31°	0.5
V	11.3	15.5	1:0.89:...	...	...
Durum (Mindum)					
Unfractionated	...	17.9	1:0.80:0.39	...	...
I	21.9	2.1	1:1.40:...	−156°	5.5
II	6.8	6.4	1:1.33:...	...	...
III	13.1	12.8	1:0.61:0.30	...	...
IV	47.4	22.9	1:0.23:0.72	−35°	0.9
V	10.9	17.7	1:1.14:...	...	...

[a]Fractionation of pentosans performed on DEAE-cellulose 23.

TABLE IV. DEAE-CELLULOSE PENTOSAN FRACTIONS[a]

Fraction	Yield %	Protein %	Ratio Arabinose: Xylose:Galactose	$[\alpha]_D^{25}$	[n]
HRS (Chris)					
Unfractionated	...	41.2	1:0.58:0.63	...	...
I	16.3	1.7	1:1.55:...	−112°	5.0
II	trace	...	...	...	...
III	18.8	26.7	1:0.25:0.98	−109°	1.2
IV	44.8	36.1	1:0.14:0.95	−34°	0.6
V	20.0	58.2	1:1.07:...	−52°	0.6
HRS (1812)					
Unfractionated	...	23.1	1:0.76:0.66	...	...
I	23.8	1.1	1:1.70:...	−131°	4.2
II	4.3	12.7	1:1.37:...	...	...
III	20.4	14.9	1:0.12:0.80	−88°	0.5
IV	34.2	34.8	1:0.15:0.92	−76°	0.5
V	17.3	56.8	1:1.09:...	−67°	0.9
HRS (Semidwarf)					
Unfractionated	...	43.7	1:0.46:0.70	...	...
I	18.8	3.5	1:1.45:...	−153°	4.3
II	3.9	14.9	1:1.13:...	...	...
III	13.0	22.3	1:0.30:0.85	−70°	0.9
IV	42.6	29.7	1:0.07:0.70	−38°	0.4
V	21.6	61.3	1:1.26:...	...	0.6

[a]Fractionation of pentosans performed on DEAE-cellulose 23.

Fraction I also exhibited the highest intrinsic viscosity in all cases. The durum gluten pentosans exhibited a higher intrinsic viscosity when compared to the HRS gluten pentosans, which may be indicative of a higher molecular weight.

The pentosans associated with the soft wheat flour gluten were similar to the HRS gluten pentosans.

In summary, the pentosans associated with gluten are similar to water-soluble pentosans. Certain differences, however, as mentioned above, do exist. Also, differences appear to exist between durum gluten pentosans and HRS gluten pentosans.

Acknowledgment

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Literature Cited

1. LIN, F. M., and POMERANZ, Y. Characterization of water-soluble wheat flour pentosans. J. Food Sci. 33: 599 (1968).
2. NEUKOM, H., PROVIDOLI, L., GREMLI, H., and HUI, P. A. Recent investigations on wheat flour pentosans. Cereal Chem. 44: 238 (1967).
3. MEDCALF, D. G., D'APPOLONIA, B. L., and GILLES, K. A. Comparison of chemical composition and properties between hard red spring and durum wheat endosperm pentosans. Cereal Chem. 45: 539 (1968).
4. COLE, E. W. Isolation and chromatographic fractionation of hemicelluloses from wheat flour. Cereal Chem. 44: 411 (1967).
5. MEDCALF, D. G., and GILLES, K. A. Structural characterization of a pentosan from the water-insoluble portion of durum wheat endosperm. Cereal Chem. 45: 550 (1968).
6. PENCE, J. W., ELDER, ANGELINE H., and MECHAM, D. K. Preparation of wheat flour pentosans for use in reconstituted doughs. Cereal Chem. 27: 60 (1950).
7. PENCE, J. W., ELDER, ANGELINE H., and MECHAM, D. K. Some effects of soluble flour components on baking behavior. Cereal Chem. 28: 94 (1951).
8. TRACEY, M. V. The role of wheat flour pentosans in baking. I. Enzymatic destruction of pentosans in situ. J. Sci. Food Agr. 15: 607 (1964).
9. CAWLEY, R. W. The role of wheat flour pentosans in baking. II. Effect of added flour pentosans and other gums on gluten-starch loaves. J. Sci. Food Agr. 15: 834 (1964).
10. TAO, R. P., and POMERANZ, Y. Water-soluble pentosans in flours varying widely in bread-making potential. J. Food Sci. 32: 162 (1967).
11. KULP, K. Pentosans of wheat endosperm. Cereal Sci. Today 13: 414 (1968).
12. D'APPOLONIA, B. L., GILLES, K. A., and MEDCALF, D. G. Effect of water-soluble pentosans on gluten-starch loaves. Cereal Chem. 47: 194 (1970).
13. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. AACC Approved methods (formerly Cereal laboratory methods, 7th Ed.). The Association: St. Paul, Minn. (1962).
14. SIMPSON, F. J. Microbial pentosanases. I. A survey of microorganisms for the production of enzymes that attack the pentosans of wheat flour. Can. J. Microbiol. 1: 131 (1954).
15. DISCHE, Z. Color reactions of pentoses. In: Methods in carbohydrate chemistry, vol. I, ed. by R. Whistler and M. L. Wolfrom. Academic Press: New York (1962).
16. LOWRY, O. H., ROSEBROUGH, NIRA J., FARR, A. L., and RANDALL, R. J. Protein measurements with the Folin phenol reagent. J. Biol. Chem. 193: 265 (1951).
17. TREVELYAN, W. E., PROCTOR, D. P., and HARRISON, J. S. Detection of sugars on paper chromatograms. Nature 166: 444 (1950).
18. CROWELL, E. P., and BURNETT, B. B. Determination of the carbohydrate composition of wood pulps by gas chromatography of the alditol acetates. Anal. Chem. 39: 121 (1967).

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