THE CARBOHYDRATES OF THE GRAMINEAE
XI. The Constitution of the Water-Soluble Polysaccharides Derived from Bread Crumb[1]

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ABSTRACT

The so-called "soluble starch" of bread consists of starch and a pentosan. "Soluble starch" from fresh bread represented, on a dry weight basis, 4.3% of the crumb and contained 11.7% pentosans; whereas, in stale bread from the same lot, the "soluble starch" represented 3.3% of the crumb and contained 19.3% pentosans. Water-soluble pentosans present in the "soluble starch" inhibited the retrogradation of amylose. Acetylation of the "soluble starch" fraction followed by fractional precipitation of the resulting acetate yielded a fraction, $[\alpha]_D^{27} - 44.5°$ in pyridine, which upon deacetylation and hydrolysis gave a polysaccharide complex containing xylose, arabinose, and glucose in the mole ratio five to four to three (5:4:3) respectively. This pentosan-rich fraction consumed 0.87 mole of periodate per pentose residue with the formation of formic acid corresponding to an average "repeating unit" of about 9.

The pentosan fraction was methylated via the acetate and fractionally precipitated. Hydrolysis and quantitative analysis of the resulting glycosides by the phenol-sulfuric acid procedure showed D-xylose (1 mol.), 2-*O*-methyl-D-xylose (1 mol.), 2,3-di-*O*-methyl-D-xylose (2.7 mol.), and 2,3,5-tri-*O*-methyl-L-arabinose (1.7 mol.). These methylation results show that the pentosan in the water-soluble polysaccharides of bread crumb possesses a highly branched structure and that it is structurally similar to the pentosan in the original flour.

It has long been known that a white amorphous powder, commonly called "soluble starch," can be isolated from bread crumb by extraction with cold water and precipitation with ethanol. The amount of "soluble starch" which can be obtained from bread crumb usually decreases upon storage (17) and, since "soluble starch" has a low iodine affinity, it has been postulated that the staling process involves association of amylopectin (26).

Unfortunately, it appears that to date only physical testing methods have been employed for the evaluation of "soluble starch" (8). The commonly employed iodine sorption (6) and fractional precipitation (7) techniques are not adequate for ascertaining the relative homoge-

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neity or heterogeneity of carbohydrate systems (14). Moreover, it appears that no chemical analysis of "soluble starch" has heretofore been undertaken.

The present paper is concerned with a study of the chemical composition and structure of "soluble starch" fractions obtained from fresh and stale bread.

Methods and Materials

Micro-Kjeldahl. The micro-Kjeldahl determination combined the digestion with selenium oxychloride (23) with the distillation and titration procedure of Ma and Zuazaga (18); perchloric acid was omitted from the digestion.

Pentosan. Total pentosans were determined by the procedure of Bailey (4).

Sugars. Sugar determinations were conducted by the phenol-sulfuric acid method (10) or the ferricyanide procedure (15).

Polysaccharide Composition. A 20-mg. sample was sufficient for hydrolysis, deionization, and chromatographic analysis as described by Gilles and Smith (14).

Acetyl Value. The acetyl value of acetylated polysaccharides was determined by the alcoholic alkali method (11).

Methoxyl Value. The methoxyl value of methylated polysaccharides was determined by the Zeisel volumetric method (5).

Methylation. Initial methoxyl groups were introduced by the modification of the Haworth procedure as applied to cereal pentosans (12,19,20). To effect complete methylation the Purdie method (25) was applied to partially methylated products which had been treated by the Haworth procedure.

Acetylation. The wheat gum was dispersed in pyridine and acetic anhydride by vigorously agitating the reactants in a Waring Blendor. After standing at room temperature until the heat of reaction had been dissipated, the solution was poured into ice water and the precipitated acetate was collected by filtration, washed with water to remove pyridine and acetic acid, and dried by suction.

Fractional Precipitation. Petroleum ether (b.p. 30°–60°C.) was added with stirring to a solution of the pentosan acetates in the least amount of acetone.

Deacetylation. The acetylated wheat gum (1 g.) was dissolved in acetone (25 ml.) and the solution was refluxed for a few minutes in the presence of aqueous 15% sodium hydroxide solution (20 ml.). The solution was cooled, acidified with glacial acetic acid, and poured into methanol. The deacetylated product was purified by reprecipitation

from aqueous solution with ethanol in the usual manner (yield, 0.5 g.).

Ash. The "straight" ashing method was used (3).

Periodate Oxidation. The method of Abdel-Akher and Smith (1) was used for periodate oxidation studies on the deacetylated pentosans.

Micropipets. Micropipets were used to apply the sugar solutions to all chromatograms used for quantitative analysis. These pipets were made from small-bore tubing, the tips being ground flat with carborundum dust on a glass plate; the pipets were standardized to deliver with constant-boiling hydrochloric acid.

Phenol Reagents. Reagent grade phenol was distilled in a glass apparatus, and stored in a dark Pyrex bottle in the dark. Suitable dilutions were prepared with distilled water as needed.

Chromatographic Sprays. For general detection of the reducing sugars, a spray of ammoniacal silver nitrate (21) or *p*-anisidine (16) was used. For methylated sugars, sprays of *p*-anisidine or N,N-dimethyl-*p*-aminoaniline (16) were used.

Water-Solubles from Wheat Flour. Water-solubles were prepared by extracting unbleached and unbromated hard wheat flour (1 kg. Southwest Bakers' Patent) with two portions (2.5 liters each) of distilled water at 5°C. under a nitrogen atmosphere. The combined extracts were centrifuged and dried from the frozen state. This water-soluble material (16.7 g.) was dissolved in 250 ml. of distilled water and the solution was dialyzed at 5°C. against five changes of water for 5 days. The solution containing the nondialyzable material was centrifuged (1.9 g. of insoluble residue) and the supernatant made up to 300 ml. (solution A). An aliquot (50 ml.) of solution A when treated with ethyl alcohol gave a precipitate which when washed and dried amounted to 1.0 g.

"Soluble Starch" from Fresh and Stale Bread. The "soluble starch" was extracted from bread crumb (1 kg.) with two portions (3 and 2 liters, respectively) of distilled water at 30°C. The combined extracts were centrifuged, concentrated under reduced pressure to about 9 liters, treated with absolute ethanol (38 liters), digested 1 hour in a steam bath, and allowed to stand overnight. The precipitated product was filtered, washed with absolute ethanol, and dried *in vacuo*.

Samples of "soluble starch" were prepared from "stale" and "fresh" bread. The "stale" sample was derived from a commercial white crumb which had been baked without the aid of emulsifying agents and had been stored for 5 days at 5°C. The "fresh" sample was extracted 2 hours after baking from the same batch of commercial bread (7). Both samples of "soluble starch" were white amorphous powders, sparingly soluble in water, giving a positive pentosan test (pink color) when

boiled with 12% hydrochloric acid solution and tested with aniline acetate paper; they also gave a blue color with iodine.

Results

Chemical Composition. General chemical tests were applied to characterize the "soluble starches." The properties and composition of the so-called "soluble starch" from fresh and stale bread are recorded in Table I.

TABLE I

ANALYTICAL DATA DESCRIBING THE "SOLUBLE STARCH" EXTRACTED FROM "FRESH" AND "STALE" BREAD CRUMB

ANALYTICAL TEST	"SOLUBLE STARCH"	
	"Fresh" Bread	"Stale" Bread
$[\alpha]_D^{25}$ *N* NaOH (c, 1)	+87.2°	+24.3°
Pentosan (%)	11.7	19.3
Nitrogen, mg/g	8.78	12.51
Ash (%)	4.94	7.20
Sugar components (by chromatography)	Glucose, arabinose, xylose	Glucose, arabinose, xylose
Percent of total crumb (dry wt. basis)	4.33	3.27

Both "fresh" and "stale" samples of "soluble starch" gave upon hydrolysis D-glucose, L-arabinose, and D-xylose. A significant point of difference in these "soluble starches" was observed in the specific rotation of their aqueous solutions. The "soluble starch" from "fresh" bread had $[\alpha]_D^{25} + 87.2$, whereas that from "stale" bread had $[\alpha]_D^{25} + 24.3$. Moreover, the "soluble starch" from "fresh" bread contained less pentosan (11.7%) than did the "soluble starch" from "stale" bread crumb (19.3%).

Samples of "soluble starch" were hydrolyzed and chromatogramed and the component sugars determined by the phenol-sulfuric acid procedure (10). The hydrolyzed "soluble starch" fraction isolated from "fresh" bread contained D-glucose, L-arabinose, and D-xylose in the mole ratio of 5:1:1, whereas, in the same bread which had been subjected to 5 days' storage at 5°C., the mole ratios of the same three sugars were 3.3:2:1 respectively.

A similar change in the relative amounts of these monosaccharides was observed in samples of "soluble starch" extracted from bread baked with emulsifying agents which were furnished by the American Institute of Baking. The D-glucose, L-arabinose, and D-xylose mole ratio in the bread 2 hours old was 2.5:0.8:1, whereas the corresponding mole ratio in the "stale" bread sample (96 hours old) was 1.88:0.75:1.

The ratio of D-glucose to pentose in the "soluble starch" decreases during the staling process. This change apparently occurs regardless of the presence or absence of emulsifying agents and may indicate that amylose or amylopectin, or both, spontaneously retrograde as staling progresses.

Acetylation of "Soluble Starch." A sample of "soluble starch" extracted from "stale" bread, which had been stored 5 days at 5°C., was acetylated in the Waring Blendor. The acetate was isolated as a stringy mass by pouring into water and was freed from impurities by washing with water, methanol, and finally with ether. The crude product was dissolved in acetone and subjected to fractional precipitation by addition of increasing amounts of petroleum ether. A summary of these results is given in Table II.

TABLE II

FRACTIONATION OF THE ACETATE OF THE POLYSACCHARIDE FROM "STALE" BREAD

FRACTION	PET. ETHER [a] ADDED TO ACETONE SOLUTION (600 ML.)	WEIGHT	$[\alpha]_D^{22}$ (ACETONE) (c, 0.5)
	ml	*g*	
I	300	7.60	−5.5° [b]
II	90	0.15	+106°
III	150	3.00	+135°
IV	100	3.50	+133°
V	150	3.40	+143°
VI	200	1.85	+157°
VII (by evaporation of mother liquors)		0.40	+157° [c]

[a] B.p. 30°–60°C.
[b] $[\alpha]_D^{22}$ in pyridine.
[c] $[\alpha]_D^{22}$ + 146° in pyridine.

Repetition of the experiment confirmed these findings. Fraction I (combined material from two experiments) was dissolved in pyridine (200 ml.) and, after addition of acetone (75 ml.), fractionation was effected by addition of diethyl ether followed by petroleum ether in quantities shown in Table III.

This fractionation appeared to furnish two components, one pos-

TABLE III

REFRACTIONATION OF THE PENTOSAN ACETATE FRACTION I FROM "STALE" BREAD

FRACTION	SOLVENT ADDED SUCCESSIVELY TO ACETONE-PYRIDINE SOLUTION [a]	WEIGHT	$[\alpha]_D^{27}$ PYRIDINE (c, 1.0)
		g	
A	600 ml. diethyl ether	4.45	−28°
B	25 ml. pet. ether	5.30	−44.5°
C	50 ml. pet. ether	1.00	+123°
D (by evaporation of mother liquors		2.20	+104°

[a] Acetone, 50 ml.; pyridine, 200 ml.

sessing a negative specific optical rotation, the other a positive rotation. Inasmuch as a study of barley gum (13) had shown that the pentosan components were concentrated in fractions with a negative specific rotation, fraction B was chosen for further work on the structure of the pentosans of "soluble starch."

Examination of Fraction B of the Acetate of the Pentosan Fraction. A sample of fraction B of the acetate was deacetylated and purified by reprecipitation. The deacetylated fraction B was hydrolyzed with *N* sulfuric acid and subjected to chromatographic analysis in the usual manner. By means of the phenol-sulfuric method, it was found that the molar ratio of the component sugars glucose:arabinose:xylose was 0.6:0.8:1.0.

In periodate oxidation studies, 0.87 mol. of periodate was consumed per "pentose" unit with the liberation of 1 mol. of formic acid per 8.8 mol. of pentose.

Methylation of "Soluble Starch" from "Stale" Bread. (a) *Haworth method.* A portion (4 g.) of the acetylated "soluble starch" fraction B was dissolved in a mixture of acetone (50 ml.) and 1,4-dioxan (125 ml.). The solution was subjected to the Haworth methylation procedure and the product collected by centrifugation.

After the second methylation the product was recovered by filtering through linen on a Büchner funnel and washing with boiling water. As the methylation proceeded, the solubility of the product in acetone increased. In all, six methylations were applied.

To obtain the greatest possible yield of the methylated pentosan, the supernatants from the first and second methylations were dialyzed against water to remove salts, concentrated by pervaporation, and subjected to four methylations by the same Haworth procedure. The methylated polysaccharide thus obtained was combined with the product from the sixth methylation of the original pentosan and the combined mixture was methylated once more.

The methylated pentosan was washed with boiling water and dissolved in chloroform, and the solution dried over anhydrous magnesium sulfate. Upon concentration of the chloroform solution, a friable, amber-colored product was isolated (yield 1.55 g., OCH_3, 36.0).

(b) *Purdie method.* A solution of the methylated pentosan (1.5 g.) in methyl iodide (15 ml.) was refluxed in the presence of silver oxide (5 g.) to effect complete methylation. The methylated product was recovered in the usual manner (25). In all, four Purdie methylations were applied. The methylated pentosan from "soluble starch," yield 1.2 g., was a friable, glasslike substance which showed $[\alpha]_D^{25} - 119.5°$ in acetone (c, 2.1). Found: OCH_3, 38.6. The methylated pentosan gave

a very faint mauve color with iodine.

Fractionation of Methylated Pentosan. The methylated pentosan (1.2 g.) was dissolved in acetone (65 ml.) and subjected to fractional precipitation with petroleum ether in the usual manner. The fractions derived from the primary fractionation were as follows:

Fraction	*Weight* mg	$[\alpha]_D^{26}$ *Acetone*
1	306.6	−93.0
2	550.3	−154.5
3	171.2	−147.0
4	158.4	±0.0

Fractions 1, 2, and 3 were then refractionated in the same manner to give the following subfractions:

Fraction	*Weight* mg	$[\alpha]_D^{26}$ *Acetone*	*Solvent Precipitation Data Ratio of Acetone: Pet. Ether*
1A	145.4	−66°	Insoluble in 1:0.7
1B	142.2	−122°	Soluble in 1:0.7
2A	14 (approx.)	...	Insoluble in 1:0.8
2B	535.4	−153°	Soluble in 1:0.8
3A	143.7	−150.5°	Insoluble in 1:2.0
3B	27.4	−67°	Soluble in 1:2.0

Fractions 2B and 3A were combined, giving a composite sample which showed $[\alpha]_D^{24} - 152.5°$ in acetone (c, 3.4) and had $-OCH_3$, 38.9. A marked similarity exists between this material and the methylated pentosan derived from barley gum, which showed $[\alpha]_D^{24} - 160°$ in acetone (12).

Analysis of the Methylated Pentosan. (a) *Hydrolysis.* The methylated pentosan (0.6154 g.) was refluxed for 15 hours with 20 parts of 2% methanolic hydrogen chloride solution, after which time the rotation had reached a constant value of $[\alpha]_D^{24} + 130°$. Hydrolysis of the mixed glycosides thus formed with *N* hydrochloric acid for 20 hours gave a hydrolysate with $[\alpha]_D^{24} + 13°$ (in the hydrolysis medium).

(b) *Chromatographic analysis.* (i) Qualitative. Partition chromatographic analysis of the neutralized hydrolysate using both 1-butanol: ethanol:water and butanone:water azeotrope revealed the presence of D-xylose, 2-*O*-methyl-D-xylose, 2:3-di-*O*-methyl-D-xylose, and 2:3:5-tri-*O*-methyl-L-arabinose, the R_f values of which are given in Table IV.

(ii) Quantitative. The analysis of the components separated on chromatograms irrigated either with 1-butanol:ethanol:water or with butanone:water azeotrope determined by the phenol-sulfuric acid procedure (10) in the usual manner was as follows: D-xylose (1 mol.), 2-*O*-methyl-D-xylose (1 mol.), 2:3-di-*O*-methyl-D-xylose (2.7 mols.), and 2:3:5-tri-*O*-methyl-L-arabinose (1.7 mols.).

Preparation of Derivatives. (a) *Isolation of components by chromatography.* The hydrolysate was placed on a sheet of Whatman No. 3

the color produced by the phenol-sulfuric acid reaction with methylated sugar derivatives having R_f values similar to that possessed by 2:3:5-tri-*O*-methyl-L-arabinose. Authentic 2:3:5-tri-*O*-methyl-L-arabinose and its glycoside displayed a maximum spectral absorption of 415 mμ; the unknown material isolated from the hydrolysate of the methylated bread pentosan, which possessed R_f 0.87, displayed a maximum spectral absorption at 415 mμ and no secondary peak, whereas authentic 2:3:4-tri-*O*-methyl-D-xylose and 2:3:6-tetra-*O*-methyl-D-glucose displayed maximum absorption peaks at 485 and 490 mμ, respectively.

Effect of Water-Solubles on Amylose Retrogradation. The water-soluble components of hard wheat flour were dialyzed to remove the low-molecular-weight components. The nondialyzable material was made up to volume (solution A). Aliquots were added to dilute aqueous solutions of amylose. The solutions were stored at 5°C. and the transmittances were read periodically at 420 mμ in an Evelyn colorimeter against water as a blank. Typical data follow:

Test Solution (5 ml. of each solution were used)	*Percent Transmittance*			
	0 hours	*17.5 hours*	*24 hours*	*114 hours*
0.4% amylose + water	97	ppt	..	..
0.4% amylose + solution A (water-solubles)	71	71	71	72
Water + solution A (water-solubles)	78	77	77	77

The data indicated that amylose precipitated spontaneously from a dilute aqueous solution. However, in the presence of the water-solubles, retrogradation of amylose was inhibited.

Discussion

The water-soluble polysaccharides, commonly called "soluble starch," which may be derived from bread crumb contain as monosaccharide building units primarily glucose, arabinose, and xylose. The term "soluble starch" is therefore a misnomer. In the so-called "soluble starch" fraction of "fresh" bread, glucose, arabinose, and xylose were found in the mole ratio of 5:1:1, whereas in the same bread which had been kept for 5 days at 5°C. the mole ratio of the three sugars was 3.3:2:1 respectively. These results show that the relative amount of pentose-containing polysaccharides in the "soluble starch" increases as bread stales and the glucose-containing component decreases. This is a consequence of the retrogradation of amylose and/or association of amylopectin as staling progresses (17,26).

These results are interesting, particularly when considered in the

light of the following facts. The total water-solubles of wheat flour, after dialysis to remove any low-molecular-weight compounds, were found to inhibit the retrogradation of a 0.2% aqueous solution of amylose at 5°C. for approximately 5 days; the control showed nearly complete retrogradation in 18 hours. Also, the water-solubles, although affecting the properties of baked bread (22), do not affect the staling rate. The "squeegee" fraction of wheat flour (19) also is said to have no effect on the staling rate. Inasmuch as the proportion of pentosan in the soluble starch from "fresh" bread is 0.51% (4.33 × 11.7) and from stale bread, 0.63% (3.27 × 19.3), it would seem that the pentosan-rich fractions of flour are not primarily involved in the staling process (see 19,20,24). Since these materials retard the retrogradation of amylose, it appears that the decrease in percentage of soluble starch which occurs during staling is mainly due to a change of state in the amylopectin. This work lends support, therefore, to the previous suggestion (26) that the amylopectin component of starch undergoes aggregation during the staling of bread.

The pentosan of "soluble starch," composed of D-xylose and L-arabinose, may be described as an arabo-xylan. From the isolation of the 2,3-di-*O*- and 2-*O*-methyl derivatives of D-xylose, it is evident that the polysaccharide is structurally related to other plant xylans and that the linear portions of the molecule are joined by 1→4 bonds. Branching, which is quite extensive since the ratio of 2-*O*-methyl-D-xylose and D-xylose to the 2,3-di-*O*-methyl derivative is 2:2.7, takes place through C_3 and through C_2 and C_3 of a relatively large proportion of the 1→4-linked D-xylose residues (see Table V). The isolation of the L-arabinose as the 2,3,5-tri-*O*-methyl derivative shows that the L-arabinose units are present in the furanose form. In addition, the isolation of only the 2,3,5-tri-*O*-methyl derivative of L-arabinose proves that all of the arabofuranose units constitute the terminal nonreducing ends in the pentosan molecule. The high negative rotation of the araboxylan of "soluble starch" shows that most of the 1→4 linkages between the D-xylopyranose residues are of the beta type, and for the same reason the L-arabofuranose units are most probably of the alpha type.

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Literature Cited

1. ABDEL-AKHER, M., and SMITH, F. The repeating unit of glycogen. J. Am. Chem. Soc. **73:** 994–996 (1951).
2. ABDEL-AKHER, M., and SMITH, F. The detection of carbohydrate esters and lactones after separation by paper chromatography. J. Am. Chem. Soc. **73:** 5859–5860 (1951).

3. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minnesota (1957).
4. BAILEY, A. J. Lignin in Douglas fir. The pentosan content of the middle lamella. Ind. Eng. Chem., Anal. Ed. **8:** 389–391 (1936).
5. BATES, F. J., AND ASSOCIATES. Polarimetry, saccharimetry, and the sugars. National Bureau of Standards, Circ. C440 (1942).
6. BECHTEL, W. G., and MEISNER, D. F. Present status of the theory of bread staling. Food Technol. **5:** 503–505 (1951).
7. BICE, C. W. A study of certain biochemical changes occurring in bread during storage. Ph.D. thesis, University of Minnesota (1950).
8. BICE, C. W., and GEDDES, W. F. Studies on bread staling. IV. Evaluation of methods for the measurement of changes which occur during bread staling. Cereal Chem. **26:** 440–465 (1949).
9. BREDDY, L. J., and JONES, J. K. N. The quantitative estimation of xylose. J. Chem. Soc. **1945:** 738–739.
10. DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., and SMITH, F. A colorimetric method for the determination of sugars and related substances. Anal. Chem. **28:** 350–356 (1956).
11. GENUNG, L. B., and MALLATT, R. C. Analysis of cellulose derivatives. Determination of total combined acyl in cellulose organic esters. Ind. Eng. Chem., Anal. Ed. **13:** 369–374 (1941).
12. GILLES, K. A., MEREDITH, W. O. S., and SMITH, F. The carbohydrates of the Gramineae. The constitution of the water-soluble polysaccharides of the grain of barley (*Hordeum vulgare*). Cereal Chem. **29:** 314–316 (1952).
13. GILLES, K. A., and SMITH, F. The carbohydrates of the Gramineae. III. A note on the nature of wheat gum. Cereal Chem. **31:** 488–489 (1954).
14. GILLES, K. A., and SMITH, F. The carbohydrates of the Gramineae. VI. The nature of the water-soluble polysaccharides of the flour of wheat (*Triticum vulgare*). Cereal Chem. **33:** 29–37 (1956).
15. HAGEDORN, H. C., and JENSEN, B. N. Zur Mikrobestimmung des Blutzuckers mittels Ferricyanid. Biochem. Z. **135:** 46–58 (1923).
16. HOUGH, L., JONES, J. K. N., and WADMAN, W. H. Quantitative analysis of mixtures of sugars by the method of partition chromatography. Part V. Improved methods for the separation and detection of the sugars and their methylated derivatives on the paper chromatogram. J. Chem. Soc. **1950:** 1702–1706.
17. KATZ, J. R. Gelatinization and retrogradation of starch in relation to the problem of bread staling. *In* A comprehensive survey of starch chemistry, ed. by R. P. Walton, pp. 100–117. Chemical Catalog Co.: New York (1928).
18. MA, T. S., and ZUAZAGA, G. Micro-Kjeldahl determination of nitrogen. A new indicator and an improved rapid method. Ind. Eng. Chem., Anal Ed. **14:** 280–282 (1942).
19. MONTGOMERY, R., and SMITH, F. The carbohydrates of the Gramineae. V. The constitution of a hemicellulose of the endosperm of wheat (*Triticum vulgare*). J. Am. Chem. Soc. **77:** 2834–2837 (1955).
20. MONTGOMERY, R., and SMITH, F. The carbohydrates of the Gramineae. VII. The constitution of a water-soluble hemicellulose of the endosperm of wheat. (*Triticum vulgare*). J. Am. Chem. Soc. **77:** 3325–3328 (1955).
21. PARTRIDGE, S. M. Filter-paper partition chromatography of sugars. I. General description and application to the qualitative analysis of sugars in apple juice, egg white, and foetal blood of sheep. Biochem. J. **42:** 238–248 (1948).
22. PENCE, J. W., ELDER, ANGELINE H., and MECHAM, D. K. Some effects of soluble flour components on baking behavior. Cereal Chem. **28:** 94–104 (1951).
23. PEPKOWITZ, L. P., and SHIVE, J. W. Kjeldahl nitrogen determination. A rapid wet-digestion micromethod. Ind. Eng. Chem., Anal. Ed. **14:** 914–916 (1942).
24. PERLIN, A. S. Isolation and composition of the soluble pentosans of wheat flours. Cereal Chem. **28:** 370–381 (1951).
25. PURDIE, T., and IRVINE, J. C. The alkylation of sugars. J. Chem. Soc. **83:** 1021–1037 (1903).
26. SCHOCH, T. J., and FRENCH, D. Studies on bread staling. I. The role of starch. Cereal Chem. **24:** 231–249 (1947).