

ISOLATION AND CHARACTERIZATION OF  
WATER-SOLUBLE PENTOSANS IN FLOURS VARYING  
WIDELY IN BREAD-MAKING POTENTIALITIES

by

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B. Sc., Taiwan Chung-hsing University (China), 1961

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A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

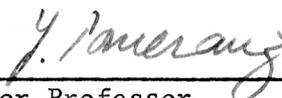
MASTER OF SCIENCE

Department of Flour and Feed Milling Industries

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1966

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## INTRODUCTION

The chemical composition of cereals has been investigated rather extensively but published data on cereal gums are limited.

Historically, an interest in this group of compounds arose only in the late thirties. Cereal gums have been studied from several points of view: their chemical composition, their relation to a number of food industries (malting, milling, bread-baking), and their susceptibility to enzymatic attack.

The effects of water-soluble gums on bread-making were studied by researchers in Switzerland, Germany, Australia and the U.S.A. Little is known on water-soluble gums in flours from different wheat varieties. This study was made to investigate the effects on bread-making of gums in flours milled from various wheat classes and varieties.

## REVIEW OF LITERATURE

History: Early work on water-soluble and insoluble gums was reviewed by Bailey (1944). The first study of water-soluble polysaccharides of cereals was reported by O'Sullivan in 1882. The pioneer work on pentosans and their estimation may be credited to Tollens and his collaborators (1890, 1891), and to Schulze and co-workers (1890).

The water insoluble gummy material recovered from wheat, as well as from barley and malt, was hydrolyzed with dilute hydrochloric acid (Lintner and Duel, 1891). Only galactose and xylose could be identified as their osazones. Wheat straw was found by Allen and Tollens (1890) to yield a water-insoluble gum which, when extracted with a sodium hydroxide solution and precipitated with alcohol, was converted into xylose on hydrolysis and on distillation with hydrochloric acid gave much furfural.

Water-insoluble hemicelluloses of wheat bran were studied by Schulze (1892). They were found to yield on hydrolysis arabinose and xylose; neither galactose nor glucose was shown.

Water-insoluble pentosans were found in whole wheat by Teller (1912) to the extent of 7.43 per cent, and in flour, bran and germ at the level of 2.60, 23.73 and 4.90 per cent respectively.

Finney (1943) separated flour into gluten, starch, and water-soluble fractions. He recombined the fractions in the original and in different proportions before baking into bread. The results demonstrated the importance of water-soluble components in bread-making. Baker et al. (1943) observed the formation of an irreversible gel upon treatment of pentosans with certain oxidizing agents used as bread improvers. This suggested soluble pentosans might have an important effect on dough properties.

Pence et al. (1950) reported that adding the water soluble fraction of flour to starch-gluten doughs from several flours gave a varied but generally large positive loaf volume response.

In 1953, Meredith et al., isolated water-soluble gums by various procedures from barley. They obtained preparations with extremely high viscosity in water. The gum-like substances were presumed to be high-molecular weight compounds.

Recent work on pentosans was reviewed by Bains (1960), Gilles (1960), Pomeranz (1961), Aspinall and Greenwood (1962), and by Neukom et al., (1962).

Water-soluble and water-insoluble pentosans: The insoluble pentosans were found similar in chemical composition to the soluble components. The differences in their physical properties were ascribed to higher branching, molecular shape, and physical entanglement of the molecules (Montgomery and Smith, 1955).

Gilles (1960) proposed classifying the pentosans and hemicelluloses (water-insoluble pentosan) on the basis of the method employed in their isolation.

The water-insoluble pentosans were found associated with the "squeegee" starch tailings obtained during wheat-starch manufacture (Clendenning and Wright, 1950). Baker et al. (1943) reported that the water-insoluble pentosans were intimately associated with the cellulose of endosperm and cell walls, and showed only very limited swelling in water. The hemicelluloses, which occur as constituents of the cell wall polysaccharides, were not water-extractable but could be extracted with cold or hot alkaline solutions (Wolf et al. (1953).

Sandstedt (1961) reported that tailings contained small starch granules, damaged starch, lipids, enzymes and insoluble pentosans. The tailings increased water absorption and the absorption tolerance, but lowered bread volume.

Wolf et al. (1953) pointed out that there was no statistical relationship between the yield of the water-soluble and water-insoluble hemicelluloses. Gilles and Smith (1954) indicated that the cereal pentosans and hemicelluloses were mixed polymers of five and six carbon sugars.

Occurrence of wheat pentosans: Bailey (1944) indicated that there were two general methods for isolating gums from cereal products, yielding different types of gums: (a) Extraction of flour or meal with water or dilute neutral salt solutions. (b) Extraction with dilute sodium hydroxide. The latter method yielded a product designated by many authors as "hemicellulose." Freeman and Gortner (1932) recovered 4 g. gum from 1500 g. of a clear grade wheat flour. Baker et al. (1943) found 2-3% pentosan in wheat flour. Pence et al. (1950) obtained between 5-8 g. of pentosans from 1 kg. of straight grade flour. The preparations contained 70-90% pentosans and up to 5% protein. According to Gilles (1952), pentosan-rich polysaccharides may represent about 4% of the wheat flour.

Flour pentosans were located mainly in the cell walls of the endosperm cells (Baker et al., 1943). About 20 to 25% of wheat flour pentosans were water-soluble (Hoffman and Gortner, 1927). Smith and Montgomery (1955) found non-starchy water soluble polysaccharides in all seeds of the Gramineae. Neukom and coworkers reported (1962) that the wheat kernel contained an average of 7 to 8% of water soluble pentosan, located primarily in the bran. In rye flour, about 40% of the pentosans are water soluble. Typical rye pentosans were water-insoluble (Baker et al., 1943).

Clendenning and Wright (1950) observed that the "squeegee" fraction was rich in hydrophilic pentosans derived from the endosperm and bran. Baker et al. (1943) suggested that wheat starch granules possessed a film of absorbed pentosan and that the properties of small starch granules were affected to a great extent

by this film because of their greater surface area per unit of solids.

Preparation of pentosan: Perlin (1951) pointed out that the differences in composition of cereal gums can be attributed to one or more of the following: (a) procedures used in isolation, each type of extraction yielding a somewhat different product, (b) difficulties in characterization of individual sugars in a mixture, and (c) use of various starting materials. Gilles (1952a) reported that extraction of wheat flour with a half saturated ammonium sulfate solution produced a gum which was high in pentosan content (67%) and had  $[\alpha]^d - 108^\circ$ . Extraction with cold (3°C) or warm (37°C) water produced gums containing lesser amounts of pentosans (19.6% and 13.6%) with  $[\alpha]^d + 10.6^\circ$  and  $80.4^\circ$ , respectively.

Baker et al. (1943) extracted the pentosan of wheat flour with water in a ratio of 1:2 (flour:water) in a Waring Blender. Proteins in the cleared centrifugate were precipitated with saturated calcium sulfate and the solution was adjusted to pH 5.5 to 5.6. After centrifugation, the pentosan gums were precipitated with ammonium sulfate, and purified by exhaustive dialysis.

Pence et al. (1950) proposed heating flour extracts in a 90°-95°C water bath for 3-4 min. to prevent a rapid decrease in viscosity. They also suggested using a special filtering agent to precipitate part of adhering low molecular weight-nitrogen compounds. The procedure of Holme (1962) for the extraction of the water soluble components was similar to that of Pence et al. (1950). The extract was treated, however, with increasing concentrations of ammonium sulfate to gradually precipitate the gliadin and albumin.

Rehfeld (1960) extracted mucin from rye meal with 80% ethanol. The centrifuged supernatant was heated to 90-93°C to precipitate coagulable protein and to inactivate enzymes. Additional proteins in the supernatant were precipitated with zinc sulfate and barium hydroxide.

Bains (1960) reviewed a number of reagents that have been used to remove protein by precipitation e.g. lead acetate, ammonium sulfate, zinc sulfate, barium hydroxide, copper sulfate, sodium tungstate, sulfuric acid, trichloroacetic acid, and certain soils. Protein can be partly removed by proteolytic enzymes such as papain, pancreatin, and pepsin. Most of the starch was removed by filtration through Celite or by digestion with  $\alpha$ -amylase or salivary amylase.

Chemical composition of pentosans: The use of paper, gas-liquid, and column chromatography, and electrophoresis have greatly facilitated research work on the chemical composition and structure of the pentosans.

The term "cereal gums" refers to non-starchy polysaccharides of cereals. Cereal gums consist largely of soluble pentosans and slightly soluble hemicelluloses (water-insoluble pentosan), and of poly  $\alpha$ - and  $\beta$ -glucans. Early studies of Freeman and Gortner (1932) indicated that the gums were largely pentose polymers mixed with some protein material. Meredith and Anderson (1953) found that the water-soluble pentosans of barley and malt were associated with some nitrogen compounds.

Pence et al. (1950) examined the pentosans in aqueous extracts of wheat flour freed from protein by absorption on Filtrol. They showed that the polysaccharides were largely composed of D-xylose and L-arabinose and that they were frequently associated with D-galactose. Perlin (1951) investigated the soluble pentosan by acetylation and fractional precipitation and reported similar results.

Kuendig et al. (1961, 1961a) fractionated the water soluble nonstarchy polysaccharides of a wheat flour extract on DEAE-cellulose. Five fractions were obtained. Fraction 1 was a pure arabino-xylan. Fractions 2 to 5 were glycoproteins; The polysaccharides of fractions 2, 4 and 5 contained xylose, arabinose, and galactose, while that of fraction 3 contained only galactose and arabinose. Neukom et al. (1962) pointed out that about half of the pentosan

preparation occurred in the form of a glycoprotein, and dissociated into an araboxyylan-protein complex and an araboxylogalactan-protein complex.

Kuendig et al. (1961) found that the UV-spectrum of the glycoprotein of a flour extract showed an absorption maximum at 275  $m\mu$ . The maximum at 275  $m\mu$  was shifted to 320  $m\mu$  by the addition of borate or germanate. This indicated the presence of an o-dihydroxy group to form cross-links between the glycoprotein molecules.

Strobel and Holme (1963) studied the water-soluble constituents of flour by means of curtain electrophoresis at pH 2.7. The components were separated into four proteinaceous and one carbohydrate fraction. Three of the proteinaceous fractions were found to contain carbohydrates. The amino acid composition of these fractions was determined and revealed that the proteins were related to albumins and gliadin.

Wrench (1965) found that enzymes in snail digestive juice degraded two of the five fractions obtained when flour pentosans were chromatographed on DEAE cellulose. Both fractions were glycoproteins containing arabinose and galactose and one also contained xylose.

The total pentosan content of crude extracts of macaroni doughs ranged 27.7% to 39.0% and appeared to be associated with proteins and polyglucosans (Bains and Irvine, 1965).

Physical properties of pentosans: Physical properties of pentosans were studied by few research workers (Montgomery and Smith, 1956). Relatively little is known about the exact size and shape of polysaccharides in their native state. Physical measurements such as optical rotation, viscosity, solubility, and osmotic parameters have been used for years to characterize polysaccharides. These physical measurements support the evidence that the cereal gums possess a large, highly branched structure which is highly hydrophylic and very viscous.

Montgomery and Smith (1956) reported that the physical appearance of pentosans and hemicelluloses isolated from flour depended largely on the technique used to isolate them. They also found that barley and wheat pentosans had a high negative rotation  $[\alpha]^d$  - 100° to - 110° and their methyl ethers had highly negative rotations,  $[\alpha]^d$  - 160° to - 170°. Pentosans prepared from wheat flour had a molecular weight of about 15,000 as determined by ultracentrifugal examination (Pence et al. 1950). Osmotic pressure measurements gave an average molecular weight value of about 22,000 for a product from which large pentosan particles and proteins were removed, and 39,000 for a more crude preparation. Luchsinger et al. (1958) indicated that after dialysis, the pentosan polymers and most of the hexose polymers in barley and malt gums had a molecular weight of 5 to 10 thousand or greater.

Structure of pentosans: The structure of the soluble wheat flour pentosans was first worked out by Perlin (1951). The pentosan components were shown to contain both anhydro-L-arabinose and anhydro-D-xylose residues. The structure was determined by graded hydrolysis, methylation, and periodate oxidation. Graded hydrolysis effected preferential removal of anhydro-L-arabinose, leading to formation of an insoluble residue composed primarily of anhydro-D-xylose units. methylation analysis of the pentosans yielded 2,3,5 tri-methyl-L-arabofuranose (3 moles), 2,3 di-o-methyl-D-xylose (3 moles), 2-o-methyl-D-xylose (1 mole), and D-xylose (1 mole). Perlin concluded that the chief characteristics of the pentosans were: straight chains of anhydro-D-xylose residues, linked  $\beta$ , 1-4, to which were appended single units of anhydro-L-arabofuranose through 1,2 and 1,3 linkages, giving a highly branched structure. Perlin (1951) explained that the water solubility and high viscosity of the pentosans resulted from the arabinose side groups. These side groups prevented an association of xylan chains and were therefore responsible for the water-solubility.

Gilles (1960) suggested that, in pentosans, chains of D-xylose units were joined by  $\beta$ -glycoside bridges and ended with L-arabinose. Principal sugars found were D-xylose, L-arabinose, D-galactose, D-glucose, D-glucuronic acid, and 4-o-methyl-D-glucuronic acid. Gilles et al. (1961) found that water-soluble pentosans upon deacetylation and hydrolysis gave a polysaccharide complex containing xylose, arabinose and glucose in the mole ratio 5:4:3, respectively. When the pentosan fraction from bread crumb was methylated via acetate and precipitated fractionally, the pentosan from bread crumb possessed a highly branched structure and was similar to the pentosan in the original flour.

Podrazky (1964) found that the water-soluble polysaccharides from endosperms of cereal grains consisted of a poly- $\beta$ -glucosan component (a linear chain of  $\beta$ -D-glucopyranose residues linked 1-4, 1-3 and to a small extent 1-6) and an araboxylan component (a basal chain of 1,4 linked  $\beta$ -D-xylopyranose units with L-arabofuranose residues with 1-3 or 1-2 linkages giving short side chains). Arabinose/xylose ratios of the araboxylan fractions for rye, wheat, barley, and oats were respectively 1:1.13, 1:1.50, 1:1.08 and 1:1.63. Rye, wheat, barley, and oat araboxylans had molecular weights of 173,000, 38,800, 58,000 and 34,200 respectively.

The water-insoluble pentosan extract of Pacific Northwest soft wheat was hydrolyzed, and separated by paper chromatography into xylose and arabinose (Hale et al. 1953). The arabinose to xylose ratio ranged 0.6 to 1.7. Xylose extracted was inversely correlated to flour yield and milling score. Arabinose showed no correlation to milling behavior.

Pentosans in dough rheology: The strong hydration capabilities of wheat flour gums reported by Hoffman and Gortner (1927) and by Freeman and Gortner (1932) might partly account for the highly viscous nature of bread doughs. The gelation phenomenon was first observed by Durham (1925) and was later studied

in detail by Baker et al. (1943). Flour to which about 1% of water soluble pentosans were added formed an irreversible gel upon reaction with certain oxidizing agents used as dough improvers. This soluble-pentosan-jelling reaction and viscosity were caused by the pentosans of malt, wheat germ and bran. Those findings were recently confirmed by Tracey (1964).

Pence et al. (1951) separated pentosans from proteinaceous material in wheat flour water-soluble fractions. They showed that the pentosans were the main contributors to the viscosity of the extracts, but the presence of a pentosanase, unless inactivated, caused a rapid decrease in viscosity.

The role of the soluble pentosan fraction was confirmed by later studies. Mattern and Sandstedt (1951) stressed the importance of water soluble material in dough mixing, and Udy (1956) pointed to the high viscosity of the extracts.

Kuendig et al. (1961) explained that the gelation phenomenon was caused by oxidation of the dihydroxy grouping, followed by the formation of cross-links between glycoprotein molecules. Neukom et al. (1962) pointed out that the gelation must be brought about by formation of cross links between oxidized pentosan molecules forming an insoluble three dimensional network.

The influence of isolated gums and pentosan-rich fractions on the rheological properties of dough can be observed by simple empirical tests. Quantitation requires use of physical dough testing equipment.

Farinograph characteristics: Bechtel and Meisner (1954), Mattern and Sandstedt (1957), and Pence et al. (1951) reported that the presence of pentosans in bread dough increased water absorption and reduced mixing time. Those effects were probably due to interaction between dispersed polysaccharides and gluten proteins. Kulp and Bechtel (1963) pointed out that adding one percent of a water insoluble pentosan-rich fraction increased water absorption 5.0 to 5.6%.

Mattern and Sandstedt (1957) studied the water-soluble constituents of wheat flour and found that they were responsible to a large extent for the mixing requirement of wheat flour. The mixing time of flour was lengthened by removal of the water-soluble material. The water-soluble pentosan precipitated by ammonium sulfate had a high amide-nitrogen content indicating that its protein largely consisted of gliadin.

Bains and Irvine (1965) studied pentosans in durum. They replaced a part of semolina of top grades with the relatively insoluble pentosan-rich gummy residues isolated from low grade semolina. This treatment was found to alter significantly the rheological properties of the resulting doughs. The mixing time and the stability of macaroni doughs were increased when the pentosan-rich fraction was added at levels of 2 to 3% to semolina of higher grades. Semolina from the higher grades contained relatively lower amounts of the crude pentosan than the lower grades.

Other physical tests: Kulp and Bechtel (1963a) found no significant effect on the Amylograph and on the Extensigraph properties of adding tailings to wheat flour doughs. Gas evolution and retention were studied in doughs made with flour to which were added insoluble pentosans. Increasing the pentosan level lowered specific loaf volume and increased crumb grain coarseness.

Podrazky (1964) studied the effects of insoluble pentosans of rye flour on Amylograph characteristics. The viscosity of rye flour increased greatly by adding 0.5% and 1% level of pentosan.

Role in baking: Pence et al. (1950) found that adding soluble flour pentosans had little effect on the baking performance of doughs reconstituted without the water-soluble pentosan, but modified markedly the handling properties of the doughs. The pentosans corrected to a large degree the slack, the softness, the wet surface, and the lack of normal stickiness, when the doughs

were reconstituted with gluten and starch only. Improvement in volume, grain and texture was small.

Bechtel and Meisner (1954) found the tailing (water-insoluble pentosan) fraction had an important effect on bread staling. The tailing fraction had a higher moisture absorption than wheat starch; its presence in flour permitted the use of more water in the dough. This in turn yielded bread of higher moisture content than if the tailing fraction was absent.

Gilles (1960) reported that in soft wheat products, the water soluble pentosans or excessive amounts of water insoluble "purified tailing" tended to reduce the spread of sugar snap cookies. The pentosans improved grain, texture, and volume of cakes.

Cawley (1964) observed that adding flour pentosans to the dough made from a gluten-starch mixture resulted in a marked increase in loaf volume and improved texture. Other viscous gums such as agar, amylose, dextran, pectin, DEAE-cellulose, and insoluble arboxymethylcellulose were without effect on loaf volume. In addition, Cawley (1964) reported that the flour-soluble fraction had no effect on gas production or retention. The greater effect on volume was through increased oven spring.

Role in milling: Weswig et al. (1963) reported that the water-insoluble pentosan yield was related to grinding, tube clearance and to grinding speed, time and temperature. The correlation coefficients between the pentosan value and milling score were - 0.74 and - 0.84 respectively for selected winter and spring wheats. The correlation coefficients between pentosan and flour yield, bran weight, and bran clean-up were significant at the 1% level.

An extensive study was conducted by Wolf et al. (1952) on Pacific Northwest wheat varieties ranging from excellent to poor in milling quality. Differences in arabinose or xylose in water-insoluble hemicelluloses could not be correlated

with milling quality of the wheat varieties.

Significant inverse correlations between the amount of acid-extracted pentosans and milling score (-0.70), and flour yield (-0.75) were obtained by Elder *et al.* (1953). Hale *et al.* (1953) found that xylose content of the acid-extracted pentosans, was correlated with the milling score (-0.78) and with the flour yield (-0.68).

#### MATERIALS AND METHODS

Sources of flour: Eight hard red winter and five hard red spring wheat samples were composited by variety from equal portions of wheat. Composite samples were prepared separately for the 1963 and 1964 crop. Among the hard red winter wheats, 'Pawnee' (C.I. 11669), 'Comanche' (C.I. 11673), 'Quivira-Tenmarq x Marquillo-Oro' (C.I. 12995), 'Chiefkan x Tenmarq' (Ks. 501097), and 'Chiefkan x Tenmarq' (Ks. 501099), were from Clovis, New Mexico; Lincoln and North Platte, Nebraska; Fort Collins, Colorado; Stillwater, Cherokee, Goodwell, and Woodward, Oklahoma; Bushland and Chillicothe, Texas; and Garden City, Hays, Colby, and Manhattan, Kansas. 'Yogo' (C.I. 8033) and 'Warrior' (C.I. 13190) were from Hymore and Brookings, South Dakota; St. Paul and Crookston, Minnesota; and Huntley, Bozeman, Havre, and Moccasin, Montana. 'Karmont' (C.I. 6700) was from Huntley, Bozeman, Sidney, and Havre, Montana. Hard red spring wheats, 'Thatcher' (C.I. 10003), 'Selkirk' (C.I. 13100), 'Marquis' (C.I. 3641), 'Lee' (C.I. 12488) and 'Pilot' (C.I. 11428) were from Brookings, Eureka, Hymore, and Newell, South Dakota; Dickenson, Fargo, Minot, and Williston, North Dakota; Morris and Crookston, Minnesota; and Havre, Huntley, and Bozeman, Montana. Single samples of soft red winter, 'Seneca' (C.I. 12529); durum, 'Wells' (C.I. 13333); and white club wheat, 'Omar' (C.I. 13072), were respectively obtained from agricultural experiment stations in Wooster, Ohio; Fargo, North

Dakota; and Pullman, Washington.

The wheat samples were milled on a Miag "Multomat" mill. Certain chemical and bread-making analysis of the flour samples are given in Table 1.

In addition, a hard red winter flour was milled on an Allis experimental mill from a composite grist of several hard winter wheat varieties grown at several locations throughout the Great Plains in 1964 (RBS-65). Certain chemical and baking properties of the flour, corrected to a 14% moisture basis were as follows:

Ash (%)	Protein (%)	Bromate requirement (mg.%)	Water absorption (%)	Mixing time (min.)	Loaf volume (cc. per 100 g. flour)
0.42	12.8	3.0	62.6	$3\frac{1}{4}$	944

Source of gums: The commercial gums used in this study were two nonionic guar gums obtained from Stein and Hall Co., New York.

Source of proteins: Proteins were isolated from the composite hard red winter wheat flour with acetic acid or urea, dialyzed, and lyophilized as described by Jankiewicz and Pomeranz (1965).

Preparation of crude pentosan: Crude pentosans were isolated in duplicate from 16 flours both from the 1963 and 1964 wheats. For infrared spectra, amino acid composition, starch gel electrophoresis, and studies of effects on rheological and bread-making properties, additional large amounts of crude pentosans were isolated from 2 hard red winter, and 1 each hard red spring, soft red winter, durum, and club wheat flours from 1964 wheats. Pentosans were isolated from the flours according to the procedure of Cawley (1964), and Kuendig *et al.* (1961) with certain modifications. Fifty g. of flour and 100 ml. of water were mixed in a Waring Blender for 3 min. The batter was centrifuged for 15 min. at 21,000 g. The extract was kept for  $3\frac{1}{2}$  min. in a 90-95° C water bath, in order to inactivate the enzymes of flour, and coagulate some proteins. The extract

Table 1. Chemical composition and bread-making characteristics of flours.

Class and Variety	Flour Extraction(%)		Moisture(%)		Ash(%)		Protein(%) (N x 5.7)		Farinograph valorimeter		Baking absorption(%)		Mixing time (min)		Bromate re- quirement(mg%)		Loaf volume (cc)	
	1963	1964	1963	1964	1963	1964	1963	1964	1963	1964	1963	1964	1963	1964	1963	1964	1963	1964
<u>Hard red winter</u>																		
Pawnee	71.5	73.5	12.7	13.7	0.56	0.40	12.4	12.1	44.0	46.0	60.5	60.0	2 $\frac{1}{4}$	2 $\frac{1}{4}$	4.5	4.0	902	898
Comanche	69.3	74.5	12.0	13.0	0.48	0.41	13.2	13.0	72.0	60.0	65.5	64.0	3 $\frac{1}{4}$	2 $\frac{7}{8}$	3.0	3.0	979	960
Qv-Tm x Mql-Oro (12995)	66.9	74.8	12.6	13.4	0.48	0.42	12.2	12.9	68.0	69.0	62.5	63.0	4 $\frac{7}{8}$	2 $\frac{5}{8}$	2.0	2.5	939	983
Chiefkan x Tenmarq (Ks.501097)	73.9	73.8	12.1	13.6	0.58	0.43	12.7	13.0	40.0	58.0	66.8	65.5	1 $\frac{3}{4}$	2	5.0	5.0	877	900
Chiefkan x Tenmarq (Ks.501099)	72.4	72.4	11.9	13.7	0.53	0.45	13.4	13.2	34.0	33.0	65.8	64.0	1 $\frac{1}{4}$	1 $\frac{3}{8}$	5.0	5.0	832	873
Yogo	72.7	73.7	12.2	13.1	0.45	0.39	10.6	12.5	47.0	50.0	59.0	61.0	2 $\frac{3}{8}$	2 $\frac{3}{8}$	3.5	4.0	851	947
Warrior	74.3	74.8	11.7	13.8	0.45	0.36	12.7	11.2	58.0	60.0	66.0	62.5	2 $\frac{5}{8}$	3 $\frac{1}{2}$	2.5	2.5	950	805
Karmont	71.7	73.8	10.8	13.0	0.49	0.42	14.0	9.9	52.0	42.0	65.5	60.0	1 $\frac{7}{8}$	3 $\frac{1}{4}$	5.0	3.0	1000	828
<u>Hard red spring</u>																		
Thatcher	69.9	73.2	13.0	14.6	0.46	0.40	13.7	13.3	68.0	51.0	65.0	63.0	3 $\frac{1}{4}$	3 $\frac{3}{8}$	2.0	2.0	1000	1038
Selkirk	69.9	73.6	12.9	14.5	0.46	0.42	13.6	13.6	68.0	62.0	64.5	62.5	3 $\frac{5}{8}$	3 $\frac{1}{2}$	3.0	2.5	1059	1030
Marquis	65.1	68.6	13.0	14.6	0.46	0.47	12.9	13.5	60.0	62.0	63.5	62.5	3 $\frac{3}{8}$	2 $\frac{7}{8}$	2.0	2.5	1055	1050
Lee	67.7	72.4	12.6	14.9	0.45	0.45	14.2	13.5	68.0	56.0	66.0	63.5	3 $\frac{1}{2}$	3 $\frac{1}{2}$	1.5	2.5	1078	1010
Pilot	65.0	70.5	12.9	14.5	0.44	0.44	12.8	13.4	60.0	64.0	63.5	62.0	3 $\frac{1}{8}$	3 $\frac{1}{2}$	1.5	2.5	1053	1050
<u>Soft red winter</u>																		
Seneca	52.5	71.3	13.3	15.0	0.36	0.37	11.3	10.0	47.0	49.0	51.0	52.0	2 $\frac{1}{8}$	2 $\frac{7}{8}$	2.0	3.0	924	863
<u>Durum</u>																		
Wells	71.2	63.1	12.2	14.9	0.74	0.69	11.6	12.8	22.0	29.0	67.5	64.5	1 $\frac{1}{2}$	2 $\frac{3}{8}$	2.0	3.0	336	568
<u>Soft white (club)</u>																		
Omar	71.5	72.7	12.2	14.0	0.40	0.39	6.6	8.6	20.0	15.0	49.0	51.0	2 $\frac{3}{8}$	2 $\frac{1}{2}$	2.0	2.0	543	700

was cooled and kept in a refrigerator at 4°C for 24 hrs. About 5 g. of "Super" Filtrol S. J. -1071 were stirred-in, and the suspension allowed to stand for half an hr. The suspension was adjusted to pH 5.5 to 5.6 by adding a dilute potassium hydroxide solution. It was centrifuged again at 21,000 g. until a clear solution was obtained. The pentosan solution was dialyzed against distilled water for 5 days at 4°C, with daily changes of water. The dialyzed extract was lyophilized and stored in a refrigerator till used.

Protein content of pentosans: Protein was estimated by a standard micro Kjelhdal procedure. Four ml. of dialyzed sample solution were pipetted into a Kjelhdal flask and dried overnight in the oven at 110°C. After weighing, 5 ml. concentrated sulfuric acid and a catalyst mixture of sodium sulphate and copper sulphate were added. The digested sample was diluted to 100 ml. To 20 ml. of diluted sample solution were added 5 ml. 35% sodium hydroxide; ammonia was collected into 2% boric acid, and titrated with 0.002 N hydrochloric acid. Protein was calculated by multiplying nitrogen by the empirical factor 5.7.

Infrared spectra analyses: Infrared spectra were obtained on a Perkin-Elmer Infracord, model 137. Pentosans were tested on discs obtained by pressing a mixture of 10 mg. pentosan material and 1 g. of an optically pure potassium bromide.

Starch gel electrophoresis: Gels were prepared by heating commercial starch-hydrolysates and 3 M urea with a buffer solution (0.017 M aluminum lactate adjusted to pH 3.2 with lactic acid). The bubble-free starch suspension was poured into the electrophoretic tray, and was covered immediately with a flexible polyethylene sheet, which had been coated with a thin film of mineral oil. Dry pentosan material was dissolved individually in 0.2 ml. lactic acid buffer solution. After the starch gel cooled, 0.07 ml. of sample solution was pipetted into each of the slots. The samples were sealed with petroleum jelly

(at 45°C), then covered with a plastic paper. The plate was assembled with the apparatus in a vertical position. The platinum electrode was connected and the compartments were filled with fresh water and lactic acid buffer solution. The electrophoresis was carried out at 20 mA for 8 hr. Then the gel was released from the tray and sliced into two portions. The cut starch gel was stained with amido black 10 B dye and washed with distilled water until a clear background was obtained.

Paper chromatography for separating amino acids: (a) Protein hydrolysis:

About 40 mg. of dry pentosan material was put into a Pyrex test tube. Five ml. of reagent-grade concentrated hydrochloric acid was pipetted into the tube, and five ml. of distilled water was added later. The neck of the test tube at a point about 1.5 cm. from the end was worked to a capillary in an oxygen gas flame. Then the tube was inserted in a dry-ice-alcohol bath, and the acid sample mixture solidified. The sample was evacuated by a high vacuum mechanical pump, and the neck of the tube was sealed by an oxygen gas flame, while it was still under vacuum. The sealed, evacuated tubes were kept in an oven regulated at 110°C for 22 hr. Then the tube was chilled and stored in a refrigerator until used. The solution was filtered through fritted glass, evaporated under vacuum three times, and redissolved exactly to 3 ml. of distilled water. (b)

Column: Dowex 50 resin ion-exchange column was used for purifying amino acids. The clear hydrolysate was passed through the column, followed by 15 ml. distilled water to wash the column. 1 N ammonium hydroxide was added to displace the amino acids. Elution of the amino acids was checked with ninhydrin. (c) Two-dimensional paper chromatography was run according to the procedure of Linko (1960). Water-saturated phenol was used as the first solvent and n-butanol:acetic acid:water (63:10:27) as the second solvent. The chromatocabs were saturated with solvent for at least 24 hr. A beaker containing solvent (100 ml.)

and another containing water (100 ml.) were placed in the bottom of the cabinet. A third beaker containing about 100 ml. of sodium cyanide also was placed in the bottom of the cabinet. Whatman No. 4 chromatographic filter paper 22" x 18" sheets were used. Hydrolysate samples were spotted in a corner, 3 inches from both edges. The paper was developed in its shorter direction first. About 40 ml. of phenol solvent was used for each paper in the trough. A beaker containing 100 ml. of 0.2 N ammonium hydroxide was put in the bottom of the cabinet. Finally, a few drops of dilute sulfuric acid was pipetted into the sodium cyanide beaker and the cover of the cabinet was closed tightly. It took 8 hr. to allow the phenol solvent to run within about one inch from the edge of the paper. The sheets were allowed to dry in air for 5 hr. The paper was then turned 90 degrees. The edge of the paper was serrated and 75 ml. of n-butanol-acetic acid solvent was poured into the trough per each paper. After 10 hr. development time, the paper was taken out of the cabinet and air-dried. The paper chromatogram was dipped into 0.25% ninhydrin in 95% ethanol, and dried immediately at 90°C for 3 min. after which the chromatograms were allowed to stand at room temperature overnight. Next, the paper chromatograms were dipped into a copper solution (2 ml. saturated cupric nitrate plus 0.2 ml. 10% nitric acid made up to 100 ml. with 95% ethanol) to stabilize the color.

Farinograph study: A ten gram Micro-Farinograph bowl was employed. Six pentosans from the five classes of flour described before were added individually at the 0.2 g. level to 9.8 g. of a control flour (RBS). The absorption of the control flour determined by the Micro-Farinograph, was 66%. In one series of experiments, 0.5 mg. potassium iodate were added in addition to 0.2 g. pentosan to the composite RBS flour. Wheat flour starch, gluten, and proteins, and two samples of commercial guar gum (control N 7-7761 B 72 and control N 10-1269) were added to the RBS flour for comparative purposes.

Determination of Amylograph viscosity: Sixty g. control flour (RBS) and 1.2 g. pentosans were suspended in 460 ml. water. After shaking, the flour slurry was poured into the Amylograph bowl. The temperature was started at 30°C and increased to about 95°C at a uniform rate of 1.5°C per min.

Alveograph curves: The study was made by the procedure of Shogren et al. (1963). Fifteen g. flour (14% moisture basis), 2% pentosans (on flour basis), and optimum water were mixed in a mixing bowl to optimum consistency with 0.45 g. of shortening and 1 ml. of a 22.5% sodium chloride solution. The dough was rounded by hand and placed for 20 min. on a metal tray in the temperature-controlled chamber. The dough was transferred to the compression chamber of the Alveograph. After relaxation for 15 min., the dough was bubbled and the corresponding Alveogram was drawn. The curve was recorded, and the length, height and area were measured.

Baking test: Baking experiments were made on a micro-scale according to the procedure of Shogren and Shellenberger (1954). The formula employed for determining the baking properties of pentosans included 9.8 g. flour (RBS), 0.2 g. pentosans, 0.15 g. sodium chloride, 0.2 g. bakers' yeast, 0.025 g. 120°L malt syrup, and 0.4 g. nonfat dry milk solids. Optimum water absorption and mixing time with the straight-dough procedure and a 3 hr. fermentation at 30°C were employed. Punching and panning were performed mechanically. Baking time was 15 min. at 218°C. Loaf volumes were determined immediately after the bread was taken from the oven. After cooling, the loaves were cut and the crumb grain evaluated. The following code was employed: S: satisfactory, Q: questionable.

## RESULTS AND DISCUSSION

Yields of pentosans isolated from the various flours varied over a range of 0.4 to 1.0% (Table 2). The highest yield was from Pawnee, and the lowest from Karmont. Analysis of variance for pentosan yield is shown in Table 3a. The means of the sixteen varieties and five classes showed significant differences at 1% level. Differences among the means of eight hard red winter and five hard red spring pentosan yields were significant at the 5% level. The interaction of variety and year was significant at the 5% level. There was no difference between the means of the two crop years.

Protein content of pentosans ranged 7.4 to 26.3% (Table 2). The pentosans isolated from durum flour had consistently the lowest protein content. The variance among the means of sixteen varieties as well as the five classes were significant at the 5% level. Differences in protein contents of pentosan preparations among the eight hard red winter varieties were significant at the 5% level. There was, however, no statistically significant difference between the protein contents of the five hard red spring wheat flour pentosans. The interaction of variety and year was not significant. The amount of protein in pentosans did not differ significantly from year to year.

Pentosan yield was negatively correlated with protein concentration in the isolated pentosan ( $r = -0.55^{**}$ ). No significant correlation could be established between pentosan yield or its protein contents, and bread-making quality or protein contents of the hard red winter or hard red spring wheat flours. The established correlation and statistically significant differences must be considered as tentative in view of the relatively large differences in flour extraction and the highly empirical nature of pentosan extraction procedure.

Table 2. Yields and protein contents of water-soluble pentosans  
from various flours.

Class and Variety	Total yield (%)		Protein content (%)	
	1963	1964	1963	1964
<u>Hard red winter</u>				
Pawnee	0.78	0.79	13.43	13.17
Comanche	0.50	0.65	23.53	12.48
Qv-Tm x Mq1-Oro (12995)	0.62	0.63	16.93	14.50
Chiefkan x Tenmarq (Ks. 501097)	0.62	0.63	10.30	8.40
Chiefkan x Tenmarq (Ks. 501099)	0.57	0.64	12.97	15.59
Yogo	0.50	0.62	22.00	16.16
Warrior	0.49	0.60	14.96	12.88
Karmont	0.35	0.64	26.31	10.15
<u>Hard red spring</u>				
Thatcher	0.73	0.62	12.73	17.78
Selkirk	0.47	0.58	20.53	13.24
Marquis	0.54	0.65	15.74	17.51
Lee	0.97	0.51	13.77	18.47
Pilot	0.80	0.54	16.22	20.89
<u>Soft red winter</u>				
Seneca	0.64	0.76	19.57	18.53
<u>Durum</u>				
Wells	0.66	0.84	8.05	7.42
<u>Soft white (club)</u>				
Omar	0.68	0.62	13.78	19.32

Table 3. Statistical evaluation of results regarding  
pentosan yield and protein content.

a. Pentosan yield .

Source of variation	df	ss	ms	F
Varieties	(15)	(57.57)	3.84	3.69**
Classes	4	17.94	4.49	4.32**
Among HRW	7	25.48	3.64	2.95*
Among HRS	4	14.15	3.54	2.94*
Years	1	2.08	2.08	2.00
Variety x year	15	69.52	4.63	3.91*
Error	32	33.17	1.04	
Total	63	162.34		

b. Protein content

Source of variation	df	ss	ms	F
Varieties	(15)	(462.59)	30.84	2.88*
Classes	4	267.32	66.83	6.23*
Among HRW	7	188.20	26.89	2.51*
Among HRS	4	7.06	1.77	0.17
Years	1	24.77	24.77	2.31
Variety x year	15	9.66	0.64	0.06
Error	32	343.01	10.72	
	63	840.03		

Infrared spectra of the water-soluble pentosans isolated from various flours are shown in Fig. 1. Absorption bands in the range  $930$  to  $1400\text{ cm}^{-1}$ , typical of carbohydrates in general (Whistler and House, 1953, Lin and Pomeranz 1965), and pentosans in particular (Marchessault, 1962), were found in pentosans from all the studied flours. Differences were found in absorption bands in the  $1600\text{ cm}^{-1}$  to  $1100\text{ cm}^{-1}$  range. This range is known to show a number of absorption spectra of amides (Sutherland, 1952). The spectra of all pentosans--except from durum--show an absorption band around  $1590\text{ cm}^{-1}$ . The low concentration of protein in the water-soluble pentosan from durum probably also explains the absence of absorption bands around  $1590\text{ cm}^{-1}$ .

Fig. 2. shows results of separating proteins associated with water-soluble pentosans by vertical starch gel electrophoresis. Comparison with gluten proteins and total proteins extracted from the flours with  $0.1\text{ M}$  lactic acid showed that the proteins associated with water-soluble pentosans resembled water-soluble proteins in electrophoretic mobility. Small differences were found in concentrations and distributions of components in club or common wheat flour proteins. Durum proteins associated with the pentosans varied substantially in their electrophoretic mobility from the proteins of pentosan-rich fractions isolated from the other flours.

Fig. 3 gives a qualitative distribution of amino acids present in proteins associated with water-soluble pentosans of six flours. Visual examination of the chromatograms indicated no significant differences in amino acid compositions of the proteins. Amino acid compositions indicated a resemblance to water-soluble proteins.

Table 4 summarizes the effects on Farinograph characteristics of adding water-soluble pentosans, with and without excess potassium iodate. The pentosans substantially increased water absorption of the composite hard red winter wheat

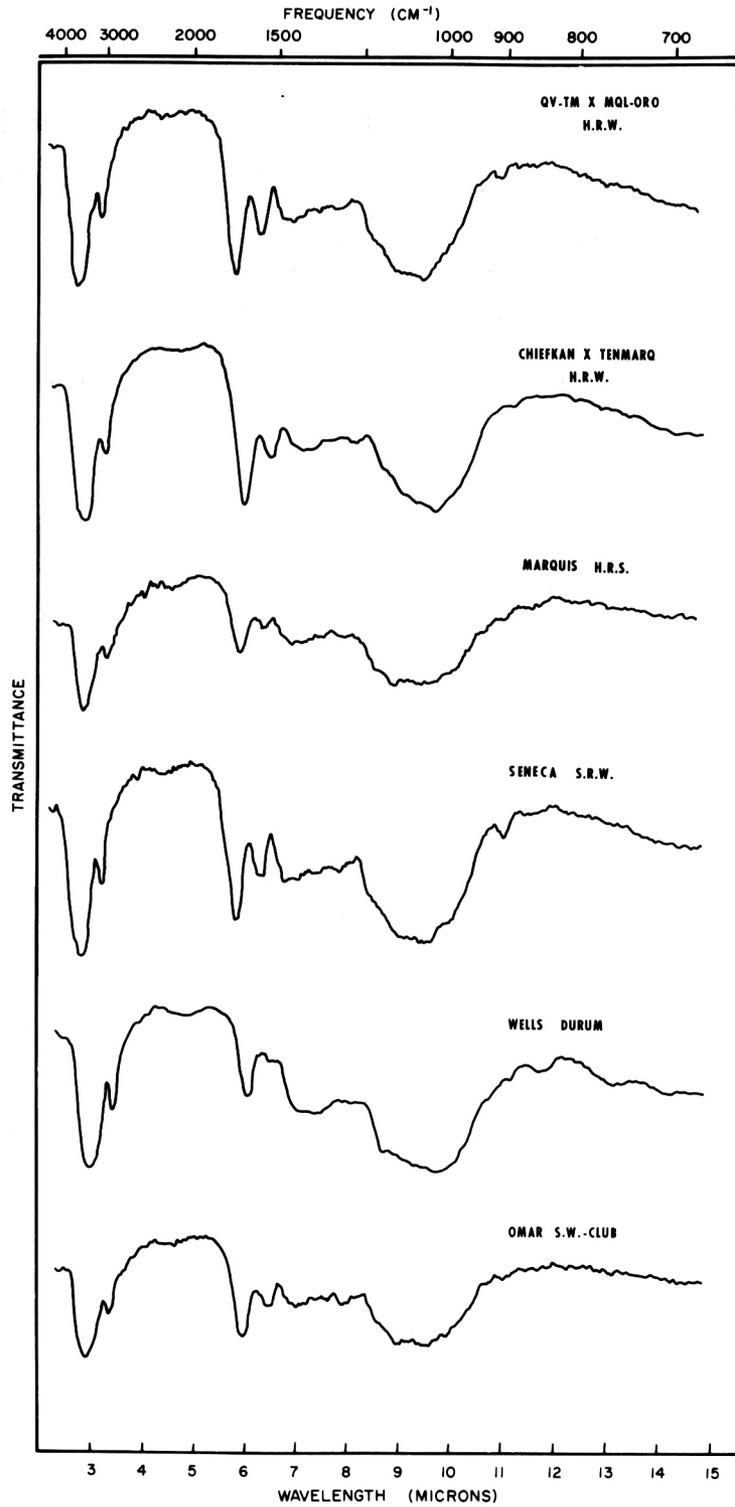


Fig. 1. Infra-red spectra of water soluble pentosans from six flours.

From left to right: Qv-Tm x Mql-Oro (HRW); Chiefkan x Tenmarq (Ks. 501099) (HRW); Marquis (HRS); Seneca (SRW); Wells (Durum); and Omar (Club). Separations of about 0.07 ml of a 3% protein solution in 3.0 M urea-0.017 M Al-lactate buffer, pH 3.2.

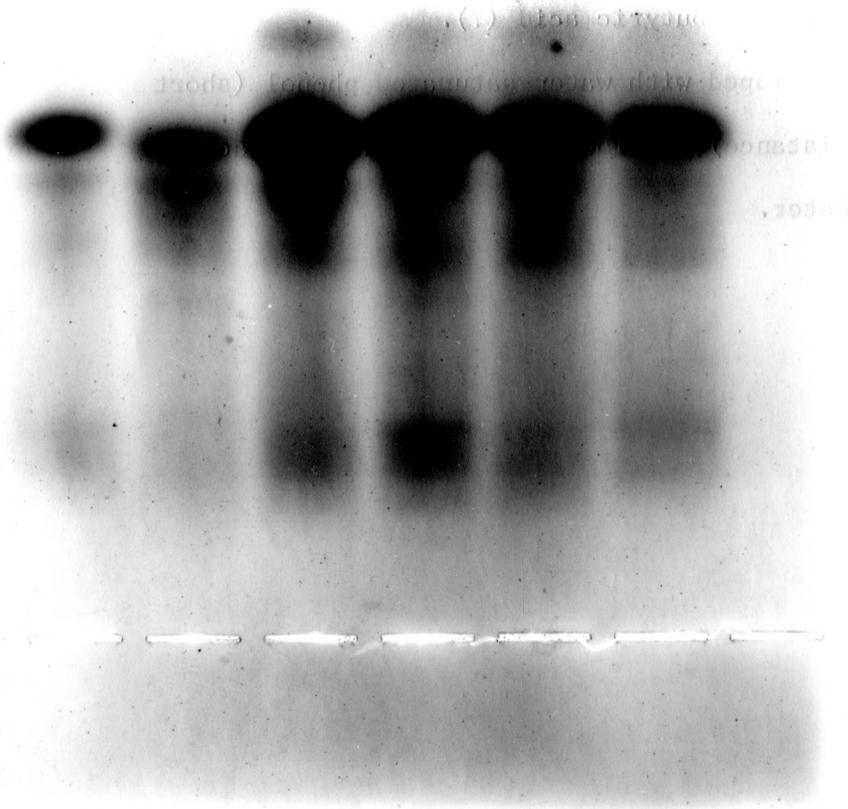
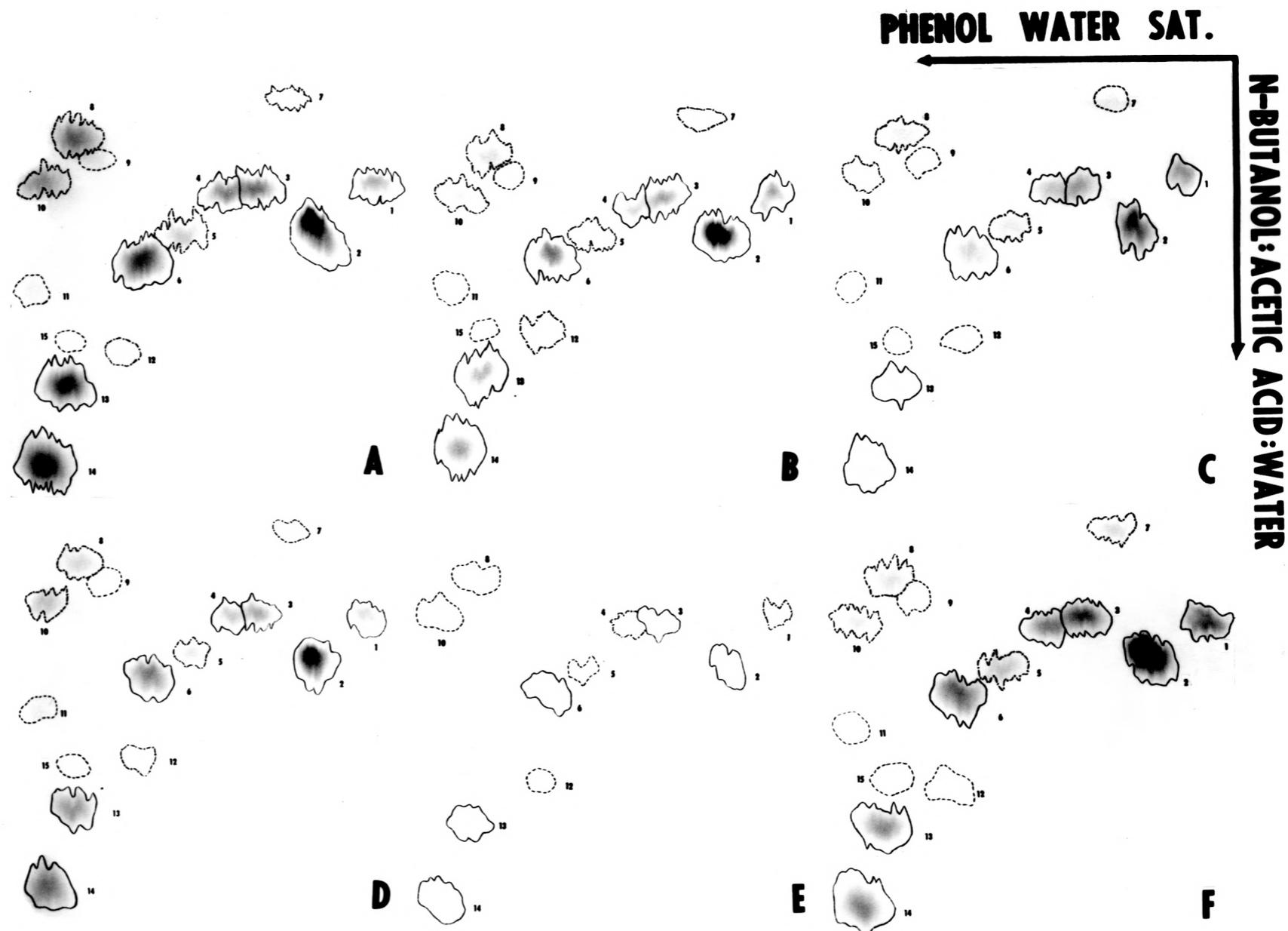


Fig. 2. Starch gel electrophoresis of water-soluble pentosans.

From: Qv-Tm x Mq1-Oro (A) (20  $\mu$ l); Chiefkan x Tenmarq (Ks. 501099) (B) (20  $\mu$ l); Marquis (C) (30  $\mu$ l); Seneca (D) (30  $\mu$ l); Wells (E) (50  $\mu$ l); and Omar (F) (30  $\mu$ l). Tentatively identified as:

1. aspartic acid;
2. glutamic acid;
3. serine;
4. glycine;
5. threonine;
6. alanine;
7. cystine (?);
8. lysine;
9. histidine;
10. arginine;
11. proline;
12. tyrosine;
13. valine, tryptophan, and methionine (?);
14. leucine and iso-leucine, and phenylalanine (?);
15. aminobutyric acid (?).

Developed with water-saturated phenol (short distance); followed by butanol:acetic acid:water.



Two-dimensional paper chromatography of hydrolysates of proteins associated with water-soluble pentosans.

Table 4. Effects of commercial gums, starch, wheat flour protein, and water soluble pentosans on Farinograph characteristics of a composite, hard red winter flour.

Ingredient added	Absorption (%)	Peak time (min)	Valori- meter value (B.U.)	Develop- ment time (min)	Stability (min)
None	66.0	5.2	52	9.7	6.2
Gluten	68.0	4.7	50	7.2	4.0
Starch	66.0	5.0	52	8.0	5.0
Acetic acid-extracted protein	67.0	5.5	52	6.7	3.2
Urea-extracted protein	66.6	5.7	55	13.0	9.7
Gum A	68.6	3.0	37	6.0	4.5
Gum B	71.8	3.0	39	6.5	5.0
Pentosan(Qv-Tm x Mql-Oro)	70.0	5.5	57	6.2	3.5
Pentosan(Chiefkan x Ten- marq, Ks. 501099)	69.8	5.2	52	7.5	4.0
Pentosan (Marquis)	69.2	5.2	53	7.5	4.0
Pentosan (Seneca)	68.8	5.5	52	7.5	3.5
Pentosan (Wells)	67.0	5.2	52	7.5	3.5
Pentosan (Omar)	69.4	5.2	53	7.2	4.0
KIO <sub>3</sub>	68.4	4.0	46	5.2	2.0
KIO <sub>3</sub> + pentosan (Qv-Tm x Mql-Oro)	71.8	4.0	49	5.0	2.0
KIO <sub>3</sub> +pentosan (Chiefkan x Tenmarq, Ks. 501099)	72.2	4.0	51	5.0	2.0
KIO <sub>3</sub> +pentosan (Marquis)	72.2	3.5	45	5.0	2.0
KIO <sub>3</sub> +pentosan (Seneca)	71.8	4.0	49	5.0	2.0
KIO <sub>3</sub> +pentosan (Wells)	66.0	4.5	49	5.5	2.0
KIO <sub>3</sub> +pentosan (Omar)	74.5	5.0	50	5.0	2.0

flour. The increase, with the exception of pentosans from durum wheat flour, was equal to, or greater than, the increase resulting from adding proteins or commercial gums. The pentosans somewhat decreased dough development time and stability. Adding pentosans, except from durum, in the presence of excess potassium iodate resulted in an additional increase in water absorption.

Table 5 shows the effects of pentosans on Alveograph characteristics. The doughs with pentosans increased Alveograph areas, length, and height consistently. Both the pentosan-free and the pentosan-containing doughs were mixed at the same level of added water. If, however, the consistency was adjusted for differences resulting from effects of pentosans on water absorption, the differences in Alveograph characteristics were small.

Table 6 shows that water-soluble pentosans had an effect on hot-paste viscosity as measured by the Amylograph. This effect was, however, much smaller than the effect found by Podradzky (1964) for rye pentosans. All the pentosans--except from durum wheat flour--added at a 2% level to a composite wheat flour, increased peak viscosity somewhat. The increase equalled or was smaller than the increase resulting from increasing the flour concentration by 2%. In the case of durum wheat flour, however, adding pentosans had a significant viscosity--reducing effect. None of the pentosans affected temperature of initial or peak viscosity.

Table 7 and Fig. 4 illustrate the effects of adding pentosans on dough and bread characteristics. The results must be interpreted with caution in view of the limitations of the micro-baking tests. The reproducibility of the results makes it possible, however, to make useful comparisons. Pentosans increased water absorption, as judged by an experienced operator. Unlike the Farinograph determination, no differences were found in development times of complete doughs mixed with a high speed mixer. The pentosans had no effect on gassing power

Table 5. Effects of pentosan and dough consistency on Alveograph characteristics.

Flour	Without pentosan			Without pentosan			Without pentosan					
	Water absorption (%)	Alveograph data			Water absorption (%)	Alveograph data			Water absorption (%)	Alveograph data		
		Length	Height	Area		Length	Height	Area		Length	Height	Area
	(cm)	(cm)	(cm <sup>2</sup> )	(cm)	(cm)	(cm <sup>2</sup> )	(cm)	(cm)	(cm <sup>2</sup> )			
RBS	62.6	25.8	1.9	16.3	62.6	25.8	1.9	16.3	62.6	29.0	2.3	17.9
Qv-Tm x Mql-Oro	63.0	19.0	3.8	25.1	60.0	13.5	5.2	28.0	63.0	22.4	4.6	33.6
Chiefkan x Tenmarq (Ks.501099)	64.0	7.8	2.5	6.0	61.5	7.2	3.6	9.4	64.0	9.3	3.8	10.2
Marquis	62.5	14.7	5.5	26.0	61.0	11.3	7.0	34.7	62.5	15.5	6.4	38.3
Seneca	52.0	10.8	4.3	26.4	50.5	10.2	9.0	38.0	52.0	7.2	9.0	29.8
Wells	64.5	3.3	7.0	12.3	64.5	3.3	7.0	12.3	64.5	3.4	8.0	15.0
Omar	51.0	5.2	4.5	10.0	48.5	6.0	5.8	10.8	51.0	3.8	6.4	12.0

Table 6. Effects of water-soluble pentosans on Amylograph viscosity of a composite hard red winter flour.

Flour wt. (g)	Water soluble pentosans (g)	Amylograph peak viscosity (B. U.)
60.0 g	None	620
58.8 g	None	570
61.2 g	None	680
58.8 g	1.2 g pentosan (HRW)	600
60.0 g	1.2 g (Chiefkan x Tenmarq)	650
60.0 g	1.2 g pentosan (HRW)	690
60.0 g	1.2 g pentosan (HRS)	640
60.0 g	1.2 g pentosan (SRW)	610
60.0 g	1.2 g pentosan (Durum)	510
60.0 g	1.2 g pentosan (Club)	660

Table 7. Effects of water soluble pentosans on bread-making characteristics of a composite hard red winter flour.

Pentosan source	Mixing time (min)	Absorption (%)	Loaf volume (cc)	Crumb grain
None	4.1	65.8	81.5	S
Qv-Tm x Mq1-Oro	4.5	68.8	83.0	S
Ks. 501099	4.0	68.2	83.5	S
Marquis	4.5	67.2	86.0	S
Seneca	4.1	67.0	81.0	S
Wells	4.4	65.5	80.0	Q
Omar	4.3	68.2	82.5	S

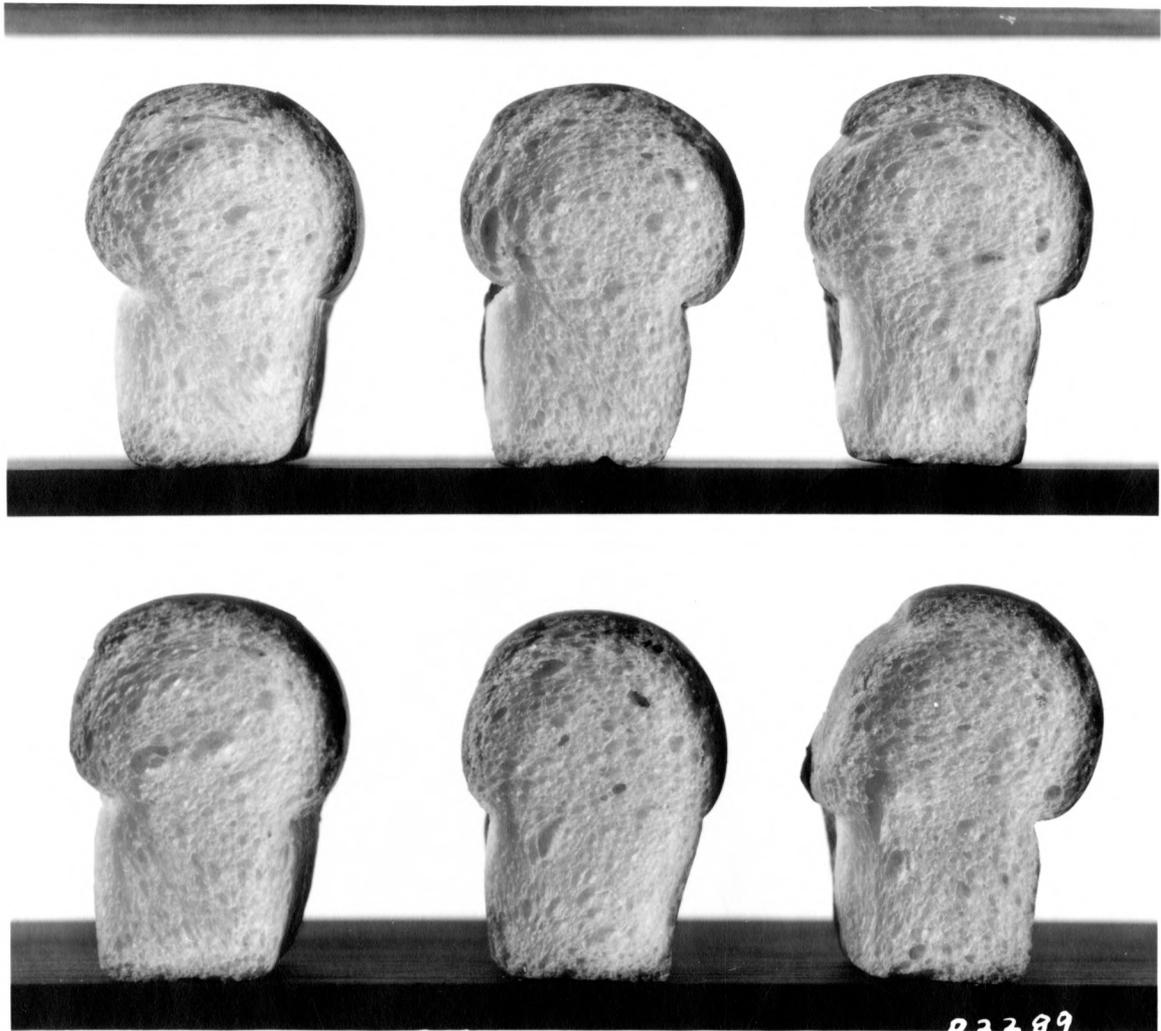


Fig. 4. Bread characteristics of micro-loaves baked from 10 g. composite hard red winter wheat flour, or mixture of 9.8 g. composite flour and 0.2 g. pentosans. From left to right; top row: control, pentosans from Qv-Tm x Mql-Oro, and from Chiefkan x Tenmarq (Ks. 501099); bottom row: baked with pentosans from Marquis, Wells and Omar.

and gas retention as measured by proof height. Loaf volumes of pentosan-containing breads were higher or equal to the control; pentosan from durum flour lowered loaf volume slightly and impaired crumb grain characteristics. Effects of pentosans on peak Amylograph viscosity (Table 6) were positively correlated with effects on loaf volume (Table 7). Additional experiments will be needed to substantiate those findings based on testing a relatively small number of samples.

## SUMMARY

Water-soluble pentosans were extracted from 16 flours. The flours were milled from 8 hard red winter and 5 hard red spring wheats composited by variety from several locations, and from single samples of soft red winter, durum, and club wheats. The pentosans were prepared separately from the 1963 and 1964 flours. The yield of crude pentosans ranged from 0.4 to 1.0%. The isolated pentosans contained 7.4 to 26.3% protein. Total water-soluble pentosan content, and protein concentration in the pentosan were significantly different in flours from various classes or varieties, but not in flours milled from wheats from the two crop years. The pentosans from durum flour from both crop years had consistently the lowest protein content.

Infrared spectra of water-soluble pentosans isolated from various flours showed differences, probably resulting from proteins associated with the pentosans. Starch-gel electrophoresis indicated that the proteins, associated with pentosans from various flours, differed both quantitatively and qualitatively. The proteins associated with pentosans were hydrolyzed by hydrochloric acid and separated by two-dimensional paper chromatography. No significant or consistent differences were found during visual observations of the chromatograms.

The pentosans from hard red winter, hard red spring, soft red winter, and from club--but not from durum wheat flours--increased water absorption. All pentosans decreased dough development time and dough stability, measured by a micro-Farinograph technique. Pentosans increased alveograph areas. A major part of this increase resulted, however, from effects of pentosans on water absorption of the doughs.

Amylograph peak viscosity was increased little or remained unchanged by adding 2% (on flour basis) of pentosans from hard red winter, hard red spring,

soft red winter, or club wheat flours. Pentosans from durum wheat flour had a viscosity-lowering effect.

All pentosans, except from durum, increased loaf volume. Pentosans from durum flour decreased little loaf volume and impaired crumb grain.

## SUGGESTIONS FOR FUTURE RESEARCH

The procedure used in this study for isolation of pentosans involved partial removal of proteins by centrifugation, heating, and use of filtering agents. The forces by which the residual proteins were bound to the pentosan isolates are, however, unknown. Pentosans isolated from wheat flour are known to constitute a heterogeneous mixture. Consequently, it would be interesting to study by fractionation on DEAE cellulose columns the composition of pentosans from various flours and the nature of proteins associated with the fractions.

Another area of interest concerns the composition of carbohydrates (pentoses and hexoses) present in pentosans from various flours and the mode of their linkage to proteins.

Pentosans have been repeatedly considered to be involved in the oxidation of wheat flours during bread-making. Use of purified pentosans from flours varying in bread-making potentialities and bromate requirements might help elucidate the role of pentosans in oxidation mechanisms.

Pentosans from durum flour differed consistently in composition and functional properties from other flours. A study of those differences, might contribute to our understanding of the differences in bread-making quality of various flours.

## ACKNOWLEDGMENTS

The author wishes to express her appreciation to Dr. Y. Pomeranz, her major advisor, for his guidance and his continuous interest in this study.

She also wishes to thank Dr. J. A. Shellenberger, Head of the Department of Flour and Feed Milling Industries for the excellent research facilities and Dr. W. E. Klopfenstein and Professor G. D. Miller, members of the advisory committee.

The author greatly thanks M. D. Shogren for micro-baking tests, and R. C. Hoseney for assistance with starch-gel electrophoresis.

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ISOLATION AND CHARACTERIZATION OF  
WATER-SOLUBLE PENTOSANS IN FLOURS VARYING  
WIDELY IN BREAD-MAKING POTENTIALITIES

by

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AN ABSTRACT OF A MASTER'S REPORT

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Flour and Feed Milling Industries

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1966

Crude water-soluble pentosans were isolated in duplicate from sixteen flours both from the 1963 and 1964 wheats. The flours were milled from eight hard red winter and five hard red spring wheats composited by variety from several locations, and from single samples of soft red winter, durum, and club wheats. The yield of pentosans extracted from the various flours varied over a range of 0.4 to 1.0%. The isolated pentosans contained 7.4 to 26.3% protein. Pentosan yield was negatively correlated with protein concentration in the isolated pentosan ( $r = -0.55^{**}$ ). Total water-soluble pentosan content and pentosan concentration in the pentosan were significantly different in flours from various classes or varieties, but not in flours from the two crop years. The pentosans from durum flour in two crop years had consistently the lowest protein content.

Infrared spectra of water-soluble pentosan isolated from various flours showed differences, probably resulting from proteins associated with the pentosans. Starch-gel electrophoresis indicated that the proteins, associated with pentosans from various flours, differed both quantitatively and qualitatively. The proteins associated with pentosans were hydrolyzed by hydrochloric acid and separated by two-dimensional paper chromatography. No significant or consistent differences were found during visual observations of the chromatograms.

Pentosans were added to a composite hard red winter wheat flour at a 2% level. The pentosans from hard red winter, hard red spring, soft red winter, and from club--but not from durum wheat flours--increased water absorption. All pentosans decreased dough development time and dough stability, measured by a micro-Farinograph technique.

The doughs with pentosans increased Alveograph areas, length, and height. A major part of this increase resulted, however, from the effects of pentosans on water absorption of the doughs.

All the pentosans--except from durum wheat flour--added at a 2% level to a composite wheat flour, somewhat increased peak viscosity as measured by the Amylograph. Pentosans from durum flour had a viscosity-lowering effect.

Pentosan-containing RBS flour was also baked on a micro-scale. All pentosans, except from durum wheat, increased loaf volume. Pentosans from durum flour slightly decreased loaf volume and impaired crumb grain.