COMPARATIVE STUDY OF PENTOSANS IN TRITICALE AND ITS PARENTAL SPECIES

A Thesis

Submitted to

The Faculty of Graduate Studies and Research

The University of Manitoba

In Partial Fulfilment

of the Requirements for the Degree

Master of Science

by

Edward Raymond Heinrichs

From the Carbohydrate Chemistry Laboratory

Department of Plant Science

Under the Supervision of

Professor R. Hill

June 1970

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to Dr. R. Hill for his guidance during the course of this research and in the preparation of the thesis.

Special thanks are also due to Mrs. L. McCrea for her technical assistance with the sugar analyses.

The financial support given by the University of Manitoba and the National Research Council of Canada is gratefully acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Heinrichs, Edward Raymond M.Sc., The University of Manitoba, June 1970.

Comparative Study of Pentosans in Triticale and its Parental Species

Major Professor - Dr. R. Hill

The pentosan compositions of one line of hexaploid Triticale (6A190), its durum wheat (T. durum cv. Stewart 63) and rye (S. cereale cv. Prolific) parents, and one hard red spring wheat (T. aestivum cv. Manitou) were determined. Triticale flour was found to contain both water-soluble and water-insoluble pentosans. On the basis of their sugar content both Triticale pentosans appeared to be more highly branched than those of its parent species. The hybrid contained about the same quantity of water-soluble pentosan as that of its durum parent. Both soluble and insoluble pentosans from the flour of all four cereals were fractionated on DEAE-cellulose columns in the borate form. Generally four fractions were obtained which differed in their carbohydrate and protein content.Triticale water-soluble and water-insoluble pentosans, and pentosan fractions had the same

degree of branching. Rye pentosans contained the most linear and most highly branched pentosan fractions.

Carbohydrate and protein distribution in pentosan fractions and sugar compositions showed that the pentosans of Triticale, as a whole, are not directly inherited from its parents.

I INTRODUCTION

Pentosans occur in all cereal grains, and flours milled therefrom, in widely varying amounts and compositions. This relatively minor but complex and heterogeneous polysaccharide, frequently described as plant cementing tissue, is closely associated with the chief structural polysaccharide, cellulose, to form the framework of the plant cell wall. Accordingly, in the kernel, most of the pentosans are located in the bran. In flour, pentosans are located mainly in the thin cell walls of the endosperm cells (1).

While typical pentosans are water-insoluble, wheat and other cereal flour pentosans can generally be classified into two types: the water-soluble, and the water-insoluble. The water-soluble pentosans are extremely hydrophilic in nature, and form very viscous solutions even in relatively low concentrations. Another unique property is their ability to form solid gels upon addition of minute amounts of oxidizing agents. Water-insoluble pentosans, on the other hand, become highly hydrated and swollen in water without going into solution.

Pentosans account for only a very small fraction of cereal flours. This highly branched polymer usually exists as a complex matrix of interwoven and mechanically entang-

led molecules, of greatly varying size, bound by the more abundant constituents such as proteins and other polysaccharides. Because of their hydrophilic properties, pentosans have a significant effect on dough properties, such as mixing time, absorption, and dough consistency.

The major difficulty in studying this group of polysaccharides lies in the fact that they have not been clearly defined. Consequently, the data and definitions have varied with the experimental procedure employed. Various attempts have been made to fractionate the pentosans into more or less homogeneous fractions. In recent years, the most successful method proved to be chromatography on diethylaminoethyl (DEAE)-cellulose columns, using a sodium borate gradient as eluant (25,31).

The purpose of this study was to examine the water-soluble and water-insoluble pentosans of a hexaploid Triticale, its tetraploid and diploid parents, durum wheat and rye respectively, and a hard red spring (HRS) wheat. Pentosans were isolated from the flours of these cereals and fractionated by DEAE-cellulose column chromatography. The chemical composition of each fraction was quantitatively determined, in an endeavour to determine from which parent or parents the pentosans of the interhybrid species, Triticale, were inherited.

II LITERATURE REVIEW

Historically, it has only been within the last thirty years that an interest has arisen in the class of compounds known as pentosans. Prior to this, only a few early descriptions of them have been recorded in the literature. Investigations of cereal pentosans have been directed primarily into three areas; their chemical composition, their relation to a series of food industries (malting, milling, baking, etc.), and their susceptibility to enzymatic attack. The following literature review will be confined to the nature and chemical composition of cereal flour pentosans.

1. Water-soluble Pentosans

Von Bibra and Ritthausen (2) isolated a pentosan which they referred to as a "gum" from rye flour by extraction with 10% sodium chloride. Ritthausen indicated that these gums were polymerized hexose sugars. Haus and Hill (3) suggested that these gums were polymers of mannose.

Gudkow (4) showed that gums from cereal grains yielded large amounts of pentose sugar. Stone and Tollens (5) confirmed this report, and isolated the phenylosazone of arabinose. Wroblewski (6) isolated a

soluble polysaccharide from wheat diastase preparation which yielded L-arabinose on hydrolysis, and which was referred to as an "araban." Geoffrey (7), who worked with the hydrolysate of a levorotatory polysaccharide prepared from an aqueous extract of flour, found arabinose only in the soluble pentose. (sic)

Hoffman and Gortner (8) extracted wheat flour with 5% potassium sulfate and obtained a gum which did not color blue with iodine, nor did it reduce Fehling's solution prior to hydrolysis by acid. This extracted material was shown to be a polymerized carbohydrate.

Freeman and Gortner (9) used a half-saturated solution of ammonium sulfate to extract pentosans from wheat flour. The analyses of these preparations showed them to have a protein content of 20% and a pentosan content of 67%. Uronic acids, galactose, and fermentable sugars, after hydrolysis, were found to be non-existent (this was also the case for pentosans from durum and rye flours). Both xylose and arabinose were identified in the wheat and rye gums. Durum gums appeared to be a pure xylan. Holme (10) reported isolation of purified pentosans (95% pentosan) by fractional precipitation with ammonium sulfate. Kulp (11) obtained a pentosan of 95-100% purity using Holme's method when special Filtrol was employed to remove residual proteins. Chromatography of acid hydrolysates of this preparation showed the presence of

arabinose and xylose in a ratio of 0.64:1.

Baker et al (12) prepared water-soluble wheat gums which contained as little as 0.1% protein. These purified water-soluble wheat gums had a pentosan content of 95% and did not contain uronic acid or galactose. Refined flour was shown to contain approximately 1% water-soluble pentosans. Baker noted that both concentrated flour aqueous extracts, and purified pentosan preparations formed a gel upon addition of sodium chlorite and other commonly used oxidizing agents.

Pense et al (13) purified an extract of wheat flour pentosans using a zinc sulfate-barium hydroxide precipitation technique. These preparations contained up to 90% pentosan and 2-3% protein. The yield was 5 g per kilogram of flour. Purification by an absorption method using special Filtrol resulted in slightly higher yields, with a pentosan and protein content of 70-80% and 3-5% respectively.

Vones et al (14) reported the composition of water-soluble gums from rye flour obtained by two procedures. The ammonium sulfate extraction procedure yielded a product which contained 37% arabinose, 61% xylose and 2% glucose. The other procedure yielded a product which contained approximately 34% arabinose, 44% xylose, 20% glucose and 2% galactose. Preece and Mackenzie (15) found that the percent yield of water-soluble pentosans, from

rye flour, as estimated by yield of furfuraldehyde was 1.05% as compared to 0.64% for wheat flour.

Gilles and Smith (16) compared the effects of three different isolation procedures upon the amounts and compositions of the recovered water-soluble pentosans from wheat flours. Using paper-partition chromatography, it was shown that all the crude wheat gums contained arabinose, xylose and glucose. Their conclussion was that the composition and content of water-soluble pentosans derived from wheat flour is greatly dependent upon the particular method of isolation.

Perlin (17) isolated the crude pentosans of flours, prepared from three different varieties of wheat, and separated them into pentosan-rich and hexosan-rich fractions by acetylation and fractional precipitation. All fractions contained both L-arabinose and D-xylose residues, and smaller amounts of D-galactose and D-glucose. The results indicated a good deal of similarity in composition between the soluble pentosans of the three flours examined. The variations in the ratio of arabinose to xylose indicated that the soluble pentosans of wheat flours comprise a series of arabinose-xylose polysaccharides. Perlin showed that the glucose polymer was distinct from the galactose component, and postulated the existence of a pentosan-galactan association.

The proposed structure of the soluble wheat flour

pentosans, worked out first by Perlin (18), explained the water-solubility and high viscosity of the pentosan solutions. The pentosan molecule consists of a backbone chain of anhydro-D-xylopyranose units, linked 1,4 probably of the beta-type, because of the high negative rotation. Attached to this chain are anhydro-L-arabofuranose residues, at the 2- and/or primarily the 3- position. These side chains prevent an association of the xylan chains, and are therefore responsible for the water-solubility. Removal of these side chains by mild acid (18) or an arabinosidase (19) result in the formation of an insoluble xylan. Using periodate oxidation Perlin found that there were approximately 300 xylopyranose residues per molecular chain. Perlin (17,18) concluded that the water-soluble pentosans from durum wheat were generally similar in structure to those from bread wheats.

This proposed structure of the basic repeating unit of the pentosan molecule was supported in more recent studies by Montgomery and Smith (20). Water-soluble pentosans were extracted from wheat flour and purified by the fractional precipitation of the acetyl and methyl derivatives. The product was composed of two parts of D-xylose to one part of L-arabinose, hence being a pure araboxylan. The cleavage products of the methylated pentosans showed that L-arabofuranose units constituted the end groups of the polysaccharide. Several workers

have established that the arabinose residues occur in the furanose form and are linked alpha-glycosidically to the xylan chain (19,21).

Preece and Hobkirk (22) have shown that the main component of the water-soluble gum fraction from rye flour is an araboxylan of similar composition to that found in wheat flour.

Aspinall and Ross (23) established that rye flour water-soluble pentosans consist of a main chain of 1-4 linked β-D-xylopyranose residues. On the average every second unit carries a single L-arabofuranose side chain, attached by a 1-3 linkage. Upon periodate oxidation it was shown that the L-arabofuranose residues are attached to isolated and less frequently, to two and three, but not more contiguous D-xylopyranose residues. The purified water-soluble pentosans used in this structural study consisted of two parts of D-xylose to one part of L-arabinose.

Preece and Hobkirk (22) reported the isolation and purification of water-soluble pentosans from whole wheat and rye by fractional precipitation with ammonium sulfate. The composition of the fractions precipitated at the 40% level for both cereals was very similar. They therefore postulated that a simple type of molecule of defined composition was present in both cereals. While pentosans extracted from wheat showed increasing solubility with

increasing araban content, rye soluble pentosans went in the opposite direction, with the most soluble fraction containing the least araban. Hence the simple Perlin hypothesis alone did not account for the phenomenum of solubility.

Pomeranz (24) suggested that not only the extent of, but also the effect of branching, and possibly the degree of polymerization were important in determining solubility of pentosans.

Kuendig and co-workers (25,26) prepared and purified water-soluble pentosans from a wheat flour. These preparations contained up to 16% protein and the polysaccharide content was made up of 41% L-arabinose, 44% D-xylose and 15% D-galactose. The pentosan preparations were completly free of glucose. Kuendig indicated, therefore, that wheat flour did not contain soluble β-glucosans and that no glucose is linked to the pentosan molecule.

Neukom *et al* (26) found that most of their water-soluble wheat flour pentosans contained small amounts of galactose and proteins which could not be separated without loss of pentosan material. It appeared that the araboxylan represented only a part of the water-soluble flour pentosans, whereas the rest was made up of much more complicated macromolecules containing proteins and galactose in addition to the pentose sugars.

Kuendig *et al* (25) applied the technique of column

chromatography, using DEAE (diethylaminoethyl)-cellulose (borate form) to fractionate wheat flour water-soluble pentosans. The purified glucose free pentosan preparation was separated into five fractions (A to E) differing in composition. Fraction A, representing about 50% of the total carbohydrate eluted was a pure arabinoxylan. Fractions B to E contained various amounts of protein and galactose. Therefore, Kuendig suggested that about half of the pentosans in wheat flour occur in the form of glycoproteins.

Kuendig stated that Fraction A, the pure arabinoxylan, was probably the end-product of preparative procedures used by some of the earlier investigators (10,17, 18,20,27). Fraction A has therefore been studied in considerable detail, however the glycoprotein fractions (B to E) had not previously been fractionated and studied.

Fraction B was the principal and most interesting glycoprotein fraction because of its sensitivity to oxidizing agents. It contained small amounts of ferulic acid which was shown to be esterified with the xylan chain, and involved somehow in gel formation. Kuendig postulated that the protein in the glycoprotein fractions was linked by covalent bonding to the pentosan since it was impossible to separate these two components by chromatography, heat sedimentation or electrophoresis.

Treatment of fraction B with Pronase, a pure proteo-

lytic enzyme, resulted in the splitting of this glycoprotein into two high molecular weight fractions (19). One of these was an arabinogalactan, containing some residual protein. This indicated that the galactose was not linked directly to the xylan chain but rather occurs as an arabinogalactan which very likely is connected via a polypeptide bridge to the arabinose of the arabinoxylan.

The exact nature of the chemical linkage between pentosans and proteins is unknown, however, and to explain the interactions, investigators have suggested at least three possibilities. The most likely linkage between protein and xylan chains is an ester bond between carboxyl groups of amino acid residues and the secondary hydroxyls of the xylose units (19,28). Other possible linkages could involve the reducing end group of the xylan chain which could form an O-glycosidic linkage with the hydroxyl groups of serine or threonine. A third hypothetical type of linkage put forward is the possible condensation between an oxidized ferulic acid residue and a reactive group such as a primary amino or sulfhydryl group from the protein part (29).

Lin and Pomeranz (30) reported the fractionation of wheat flour water-soluble pentosans on DEAE-cellulose columns according to the procedure of Neukom et al (31). The preparation and purification procedure were essentially the same as Kuendig used. The yield and protein con-

tent of pentosans from various wheat flours ranged from 0.47-0.58% and 18.9-22.6% respectively. From a durum wheat flour the yield was 0.38% and the protein 16.9%. Fractionation of a purified water-soluble pentosan from a HRS wheat flour resulted in five fractions. Fraction A was the smallest and accounted for only 1% of the total carbohydrate eluted. Fractions B to E contained a series of glycoproteins. Fraction E was the largest, in contrast to Kuendig's report, where fraction A was the largest. Fraction C had the highest protein content whereas Kuendig's fraction E was richest in protein. Lin and Pomeranz suggested the possibility of non-covalent bonding in glycoproteins, based on the observation that the protein and carbohydrate elution peaks did not coincide. The results in this study indicated the elution of a heterogeneous mixture of proteins, or of proteins and glycoproteins.

Wrench (32) fractionated a typical Australian wheat flour water-soluble pentosan, using Kuendig's procedure. Neither fractions C or D contained xylose. This was in contrast with Lin and Pomeranz's fractions C and D which both contained xylose. Kuendig's fraction D contained xylose whereas fraction C did not.

Medcalf *et al* (33) used the DEAE-cellulose chromatography procedure, as described by Kuendig, to fractionate the purified water-soluble pentosans from HRS, durum

and rye flours. For comparison, water-soluble pentosans from pearled durum and HRS wheat were isolated under a different isolation procedure, namely, acetylation and fractional precipitation. Four fractions were obtained by the acetylation procedure, and the degree of branching as measured by the arabinose-xylose ratio was about the same for all fractions from a given sample. Fractionation, according to these workers was based primarily on molecular weight differences.

The pentosan preparations used in the DEAE-cellulose procedure contained more arabinose than xylose, in contrast to previous reports. The durum pentosan preparations contained 12% protein whereas those from HRS wheat contained 17%. The latter contained the hexose sugar galactose, while the durum pentosans contained the hexose sugar glucose. Upon fractionation on DEAE-cellulose, five fractions were obtained (A to E). The essentially pure arabinoxylan fractions, A and B, appeared to be generally similar to the major fractions obtained by the acetylation procedure. Fractions C to E contained varying amounts of protein. Medcalf and co-workers showed that soft wheat soluble pentosans had a lower degree of branching than either durum or HRS wheat. Rye water-soluble pentosans had the same degree of branching as the HRS wheat sample.

Medcalf *et al* indicated that the pentosans from the water-soluble portion of durum endosperm were more highly

branched than those from HRS. This was true for pentosans isolated by both the acetylation and DEAE-cellulose procedures. The fact that both procedures led to the same conclusion is strong evidence that the observation was a real one and not an artifact of the isolation procedure.

2. Water-insoluble Pentosans

Baker et al (12) presented evidence that the insoluble wheat flour pentosans were deposited or adsorbed upon the surfaces of the starch granule. The small starch granules were found to have a surface area 30 times greater than the large granules, and contained approximately 30 times as much pentosan as did the large ones. It was suggested that this coating of insoluble pentosan could account for many of the properties of starch and for its behaviour toward enzymes.

MacMaster and Hilbert (34) investigated the composition of the "amylodextrin" or small granule fraction of wheat flours. This amylodextrin fraction comprised about 11% of the flour and was found to contain 90% starch, 1.5% protein, 4% pentosan and 3% cellulosic material. Baker et al (12) reported 14% pentosan in a comparable fraction. The composition of this crude pentosan fraction, as reported by different investigators, has varied considerably and is due to the fact that a mechanical separation is involved in removing this mucilaginous material from the prime starch. This crude insoluble

pentosan fraction has been refered to by various investigators as "amylodextrin" (35), "squeegee" (36), "tailings" (34) or "sludge" (33).

Yamazaki (37) reported a procedure for further purifying tailings of wheat flour. This was accomplished by prolonged wet sieving of tailings using a 325-mesh sieve. Chemical analyses gave rather wide ranges for components. Total nitrogen of purified tailings varied from 0.5-3%, starch from 7-23% and pentosan from 30-65%. These purified tailings had high alkaline water retentive values, sorbing from 10 to 16 times their weight of water.

By enzymatically degrading the starch entrapped in squeegee, Simpson (38) isolated the pentosan as the main constituent. Purified squeegee amounted to 0.5-1% of the wheat flour and contained 70% pentosan.

Kulp and Bechtel (39) used a 400-mesh sieve in the removal of starch from tailings. Purified tailings were found to contain 54-60% pentosan, 5% protein and 2% lipid. The remainder was mainly starch and cellulose as found by others (34,37). The yield was 15-25 g per 5.45 kilograms of flour. Pancreatin digestion increased the pentosan content only slightly. Extraction with 0.5N sodium hydroxide followed by neutralization with acetic acid and precipitation with ethanol gave a product that contained 69% pentosan and 2% protein. These workers indicated that use of strong alkali is undesirable since

there is evidence that it causes a slow degradation of the polysaccharide and rupture of complexes of pentosans with other polymeric compounds.

Perlin and Suzuki (40) fractionated purified squeegee with increasing concentrations of potassium hydroxide. All the alkali soluble fractions were found to contain arabinose and xylose as major constituents, and a lesser amount of glucose and mannose. The alkali insoluble residue, which accounted for approximately 4% of purified squeegee, was found to contain glucose, mannose, xylose and cellobiose. It was suggested that the mannan and cellulosic components might be important in contributing to the viscosity characteristics of squeegee by their ability to increase both water absorption and interfiber bonding.

Upton and Hester (41) obtained wheat flour tailings which contained 7% pentosan, 6% protein and 87% starch. These tailings were subfractionated by shearing action and high velocity centrifugation into three fractions. The second subfraction, B, had a pentosan content of 18%, which was 3 times greater than that of the two other subfractions. Subfraction B, which contained 16% protein, was further purified by treatment with two enzymes, pancreatin and a protease. The resultant product contained 95% pentosan and 5% protein. The constituent sugars of this purified product were arabinose and xylose, in the

ratio of 1:1.6 respectively. The other two subfractions contained varying amounts of glucose in addition to arabinose and xylose. It was not determined whether glucose was linked covalently to the pentosan molecule, or whether the glucose-containing fractions were mixtures of glucosans and pentosans. Each of the three subfractions obtained contained different arabinose-xylose ratios.

Graded hydrolysis showed that arabinose was released more readily from the polysaccharide than xylose. It was suggested that arabinose might be more exposed in the polymer structure, be bound to the polysaccharide polymer by a less stable bond, or be present in a more labile structural form than xylose.

The proteins of subfraction B, prior to further purification, were more similar in starch-gel electrophoresis patterns to the water-soluble proteins of the flour than to gluten. This was in contrast to the proteins in subfraction A, the supernatant subfraction of the tailings, which were more similar to gluten proteins. Upon further purification subfraction B showed little if any migration through starch gel. It has been suggested (25) that the behaviour of wheat polysaccharide-protein complexes is dominated by the polysaccharide portion and that such complexes do not give typical protein reactions.

About 20% of the total protein of subfraction B, or 65% of that of purified subfraction B, its equivalent,

was shown to be resistant to hydrolysis. This investigation strongly indicated the existence of glycoprotein fractions in the water-insoluble pentosan component. The strong structural integrity of this polysaccharide-protein complex was attested to.

Montgomery and Smith (27) investigated the composition and structure of the water-insoluble pentosans from the squeegee fraction of wheat flour. Purified squeegee was extracted with 0.5N sodium hydroxide, acetylated and fractionated. The structure of the acetone insoluble portion, which was a relatively pure hemicellulose, was investigated. This purified hemicellulose consisted of 59% xylose, 39% arabinose and 2% glucose. This water-insoluble pentosan had a higher arabinose-xylose ratio than the water-soluble pentosans similarly prepared (20). It was suggested, therefore, that the insoluble pentosans were more highly branched than the soluble ones.

It was established that water-insoluble pentosans contained arabinoxylans of similar structural features as water-soluble pentosans (27,40). The insoluble pentosans were shown to consist of a linear framework of D-xylopyranose units attached by beta 1,4 links. L-arabofuranose side chains were attached to the xylan chain principally through position 3. The differences in physical properties of the water-soluble and water-insoluble pentosans have been attributed to higher branching of the insoluble

araboxylan, differences in molecular shape, and physical entanglement of the molecules.

Medcalf and Gilles (42) recently reported the structural characterization of the main pentosan fraction isolated from the water-insoluble portion of durum wheat endosperm. The sludge portion was purified by the pancreatin digestion and alkali extraction procedures. Purified sludge pentosans yielded 0.5% and had a protein content of 1.8%. One acetone insoluble and 3 acetone soluble fractions were obtained by acetylation and fractional precipitation. The first soluble fraction was obtained in highest yield, 35%, and was used for structural characterization. This fraction contained less than 1% protein and consisted of 53% xylose, 41% arabinose and 6% glucose. The D-glucose was not considered as part of the pentosan molecule, since it was removed upon methylation.

The water-insoluble pentosans from durum endosperm, though more highly branched, were similar in structure to the pentosans from wheat flour (18,20,21,27) and rye flour (23). The durum insoluble pentosan molecule was found to be composed of D-xylopyranose units linked beta 1,4, with single unit L-arabofuranosyl branches attached to the 3- position of the xylose units, or the 2- and 3- positions of the xylose units. About 3 of every 5 xylose units were branched, and 6 of every 15 branched xylose

units contained double branches. The Smith Degradation procedure (43) was used to elucidate the distribution of branching. Branches occured predominantly on alternating D-xylose units, occasionally on 2 contiguous units and very infrequently on 3 or 4 contiguous xylose units.

Cole (44) applied the technique of column chromatography in fractionating wheat flour insoluble hemicelluloses. Purified tailings were obtained from a hard red winter wheat flour. About 80% of these hemicellulose solids could be solubilized with dilute base. Previously it had been reported that dilute base removed only about 20% of the total pentosan in tailings (45). Analyses of these hemicellulose solids were as follows: xylose 54%, arabinose 33%, glucose 11%, galactose 2% and protein 2%. DEAE-cellulose, in the borate form was found to be effective in separating the hemicelluloses into 5 fractions differing in composition. Other adsorbents such as Sephadex G-200 were ineffective, since the hemicelluloses were excluded. This indicated that these polysaccharides were either highly associated or had molecular weights exceeding 200 -thousand. The presence of such high molecular weight polysaccharides in these hemicellulose preparations also indicated that a minimum of degradation had occured during solubilization with dilute alkali. Fraction A, which accounted for 42% of the total eluted, did not complex with borate and was eluted with water.

The first four fractions contained negligible amounts of nitrogen, however, over one-third of fraction E, which accounted for 22% of the total eluted was protein. This fraction also had the highest glucose content.

Medcalf *et al* (33) fractionated the purified tailings from a HRS wheat flour and a durum semolina by the DEAE-cellulose procedure. Water-insoluble pentosans from pearled durum and HRS wheats were isolated by the acetylation procedure and fractionally precipitated. The latter method produced a purified sludge fraction which contained arabinose, xylose and glucose. The wheat pentosan preparations had an arabinose:xylose:glucose ratio of 1:1.4:1.3 whereas the durum pentosans had a ratio of 1:1.2:0.6. Of the 4 fractions obtained in each case, fractions A and B were essentially pure arabinoxylans. As was the case for water-soluble pentosans, molecular size appeared to be the major variable that controlled fractionation.

Viscosity data for the sludge fractions isolated by the acetylation procedure indicated that the durum pentosans were of slightly higher molecular weight than the corresponding HRS wheat sludge pentosans. The major difference observed between the water-soluble and sludge pentosans isolated by this procedure was the higher molecular weight of the sludge fractions.

The purified insoluble pentosans used in the DEAE-cellulose procedure contained D-xylose, L-arabinose and

D-glucose. Protein content was 7% for the durum sample and 18% for the HRS wheat sample. These purified sludge pentosans were fractionated into 5 fractions by column chromatography according to the procedure described by Kuendig et al (25). The first 3 fractions were arabinoxylans while fractions D and E contained glucose. All fractions contained protein, with fraction E containing the most. As was the case for the water-soluble pentosan fractions, fraction A was less highly branched than fraction B. Fraction C was the most highly branched of the sludge arabinoxylan fractions. This was true for both isolation procedures used.

The differences observed in this study between HRS and durum wheat pentosans were significant but relatively small. However, even small differences in degree of branching were postulated to alter markedly the degree and type of interaction of polysaccharide with protein. The pentosans from the sludge portion of the endosperm were found to be less highly branched than those from the water-soluble portion of the endosperm. The degree of branching, however, was similar for both water-soluble and sludge pentosans within each wheat type.

III MATERIALS AND METHODS

1. Flour Samples

The cereal line and varieties used in this study were: one line of Triticale (6A190), Stewart 63 durum wheat, Prolific rye and Manitou hard red spring wheat. The Triticale strain, 6A190, was produced by crossing durum wheat, T. turgidum, cv. Stewart 63 and rye, S. cereale, cv. Prolific. The rye parent contained the R genome while the durum wheat parent contained the A and B genomes. The resultant Triticale strain contained all three genomes, A, B and R.

The samples were milled into flour on the Buhler experimental mill after tempering overnight at 15.5% moisture.

2. Extraction and Purification of Water-soluble Pentosans

Water-soluble pentosans were isolated from all four flours by the same procedure, as illustrated by wheat flour in Fig. 1. A slurry of 150 g of flour and 300 ml of distilled water were placed in a Waring Blendor, mixed at high speed for 3 min and then centrifuged at 1000 x g for 30 min. The supernatant was heated to 95°C for 3½ min to inactivate any endogenous enzymes and to coagulate heat denatured proteins. The coagulum was removed by

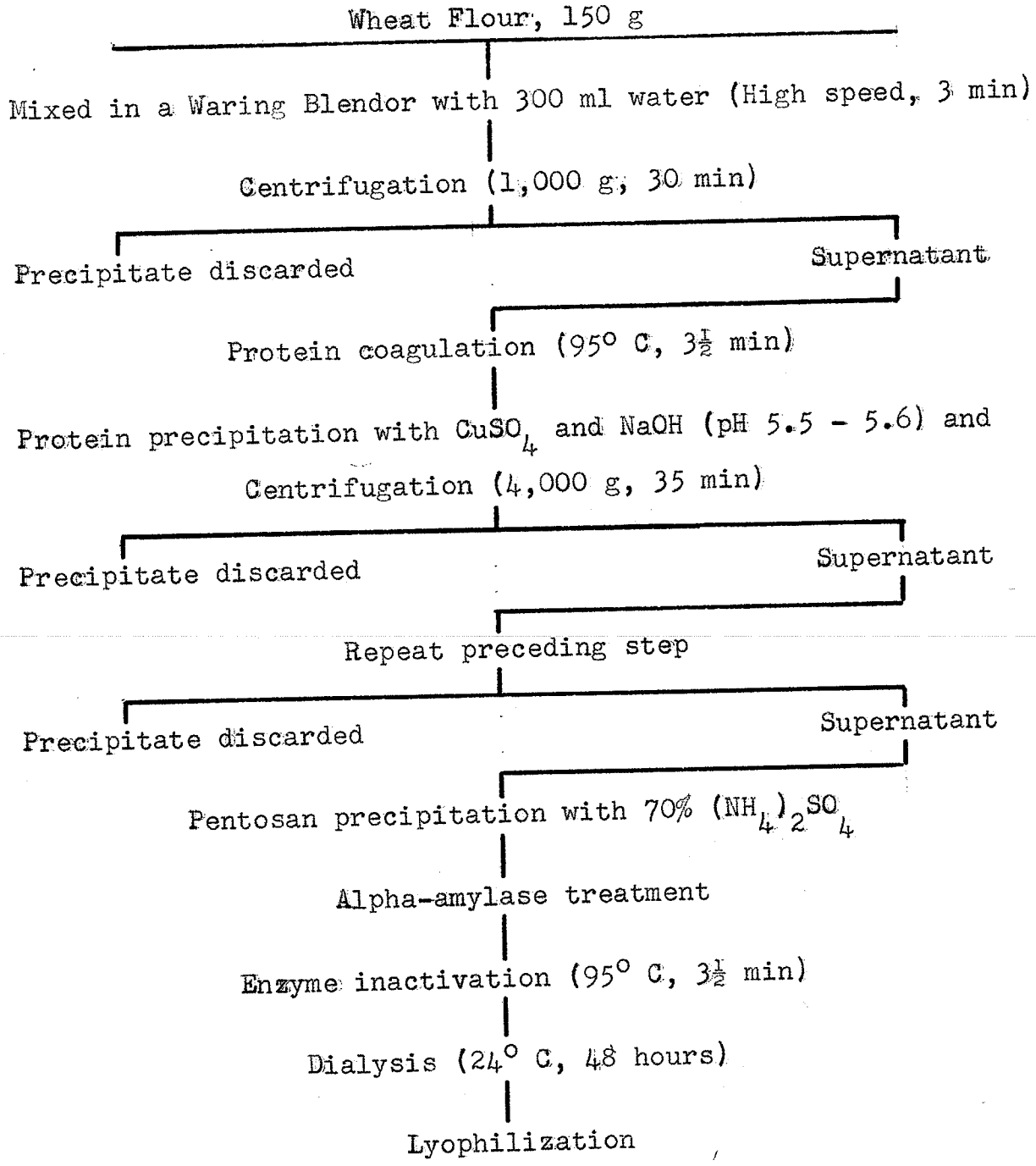


Fig. 1. Outline of preparation and stepwise purification of water-soluble wheat-flour pentosans.

filtering the supernatant through glass wool. Protein was further removed by the procedure described by Baker et al (12). Six ml of 5N sodium hydroxide and 16.7 ml of a saturated copper sulfate solution were added per 100 ml of supernatant and the mixture stirred with a magnetic stirrer for 15 min. The pH was checked and adjusted if necessary to between 5.5-5.6 using either dilute sulfuric acid or sodium hydroxide. The mixture was poured into centrifuge bottles and centrifuged at 4000 x g for 35 min. The residue was discarded. The supernatant obtained was treated once more in the manner just described. The resultant supernatant was made 70% with respect to ammonium sulfate, stirred for 15 min and placed in a refrigerator overnight. The water-soluble pentosans, which had coagulated and risen to the top of the saturated ammonium sulfate solution, were mechanically removed and allowed to dissolve in 200 ml of a 0.02 M sodium phosphate buffer, pH 7.2. Approximately 2 mg of a bacterial alpha-amylase (Sigma Chemical Co., 1 mg liberates approx. 990 mg of maltose from starch in 3 min at pH 6.9 at 20°C) were added and allowed to act on any starch or alpha-glucosan present. After 2 hours the enzyme was inactivated by heating to 95°C for 3½ min. Upon cooling the coagulated enzyme was removed by filtration and the filtrate was dialyzed against distilled water for between 3 to 5 days, or until no low-molecular weight sugars could be detected in the

dialyzing medium. A few drops of toluene were added to prevent growth of microorganisms during dialysis. Upon completion, the contents of the dialysis tubing were removed, lyophilized and designated as purified water-soluble pentosan.

3. Extraction and Purification of Water-Insoluble Pentosans

Flours from both HRS and durum wheat form a relatively stable gluten ball, and this allows for the removal of gluten or water-insoluble proteins from a flour. Three hundred g of flour and 240 ml of distilled water were added to a GRL (Grain Research Laboratory) mixer and mixed at low speed. After the dough had relaxed for 5 min, an additional 500 ml of distilled water was added and mixed for 5 min. The mixing bowl was scraped by hand to free any attached gluten after which another 100 ml distilled water was added. After mixing for 5 more min the gluten ball was thoroughly washed with distilled water and then discarded. Both the wash water and the thin slurry in the mixing bowl were poured into centrifuge bottles and centrifuged at 1000 x g for 30 min. The supernatant was discarded and the tailings layer, which appears on top of the prime starch layer, was mechanically separated. The tailings were transferred to a 400-mesh sieve and washed with distilled water by wet-sieving for at least 30 min or until a negative starch test was obtained by iodine. The tailings were subsequently treated with alpha-amylase

for 2 hours, under appropriate conditions, to remove any possible remaining starch. Enzyme activity was stopped by heating to 95°C for 3½ min. The tailings were wet-sieved for another 30 min, lyophilized and designated as purified water-insoluble pentosans.

Rye and Triticale flours do not form a gluten ball, and therefore a slightly different procedure was used in extracting the water-insoluble pentosans. Flour and water were mixed in a Waring Blendor for 3 min and then centrifuged at 1000 x g for 30 min. The supernatant was discarded and the residue in the centrifuge bottles consisted of 3 layers: prime starch, gluten proteins and starchy tailings. The tailings layer was mechanically separated and washed free of starch with distilled water by wet-sieving. The relatively large amounts of water-insoluble proteins still present were removed as far as possible by several centrifugation and mechanical separation steps. The tailings were treated with a bacterial alpha-amylase for 2 hours in a phosphate buffer to remove any remaining traces of starch. Enzyme activity was terminated by heating to 95°C for 3½ min. The tailings were washed once again with distilled water over a 400-mesh sieve to remove low-molecular weight substances, and then lyophilized. The product obtained was designated as purified water-insoluble pentosan.

4. Preparation of DEAE-Cellulose Columns

The DEAE-cellulose (Sigma Chemical Co., medium mesh, exchange capacity 0.80 meq/g) column was prepared in the borate form according to Neukom and Kuendig (46). Dry diethylaminoethyl or DEAE-cellulose was washed 3 times consecutively with 0.5N hydrochloric acid followed by distilled water, and 0.5N sodium hydroxide followed by distilled water using reduced pressure to de-gas the suspensions. Fines were removed by decanting the supernatant acid, water and base after each washing. Distilled water was used in the final washing, which was carried out on a Buchner funnel, until the passing water had a neutral pH. The cellulose was converted to the borate form by suspending the washed dry cellulose in either a 0.25M or 0.50M solution of potassium borate. The cellulose was stirred slowly by means of a magnetic stirrer and left under vacuum overnight. A fairly thin slurry was used in filling the columns, during which time the slurry was continually stirred and kept under vacuum. The columns used were 30 x 1.8 cm and were made from glass tubing. A glass wool plug was inserted at the bottom of the column. A 1 cm layer of glass beads (60/80 mesh), which had been thoroughly washed, was placed over the glass wool plug prior to adding the cellulose slurry. Either a layer of glass beads or a disc of filter paper was placed on top of the cellulose after filling. Prior to using, the excess

borate was washed off with the starting buffer, distilled water. The columns were used only once, however, the DEAE-cellulose was regenerated and re-used several times.

5. Fractionation on DEAE-Cellulose Columns

Purified water-soluble pentosans were dissolved in distilled water and then applied directly to the top of the column. In each case 3 ml of the highly concentrated pentosan solution, containing about 35 mg of polysaccharide, was applied. The purified water-insoluble pentosans were solubilized by extracting with 0.5N sodium hydroxide. Inorganic ions were removed by means of a diaflow ultrafiltration cell, using a UM-2 membrane (molecular weight exclussion limit of 1000). The water-insoluble pentosans were generally concentrated to between 6-9 mg polysaccharide per ml and then applied directly to the top of the column. After the sample had been allowed to penetrate into the DEAE-cellulose, elution was accomplished stepwise with the following eluants: (1) distilled water; (2),(3),(4) potassium borate ranging from 0.0025M to 0.50M; (5) 0.4N sodium hydroxide. The column effluent was collected in 15-ml fractions with the use of an automatic fraction collector. The flow rate of the eluate was maintained at 1.4 ml per min. The quantitative elution of polysaccharide fractions was followed colorimetrically with the phenol-sulfuric acid method of Dubois et al (47), with xylose as standard. The photometric readings were

done on a Bausch and Lomb spectrophotometer (Spectronic 20) at a wavelength of 480 millimicrons. After the positions of the individual fractions were located, the eluates which comprised the peak and major part of each fraction were combined and dialyzed against distilled water for at least 48 hours. Each fraction was subsequently concentrated in a rotary flash evaporator and stored in the refrigerator.

6. Determination of Protein Content

Protein content (N x 5.7) of the flours and the purified water-soluble and water-insoluble preparations were determined using the standard macro Kjeldahl and micro Kjeldahl procedures respectively. Protein content of the fractions obtained by the DEAE-cellulose fractionation procedure were determined by the method of Folin-Ciocalteu as modified by Lowry *et al* (48), using bovine serum albumin as standard. The Bausch and Lomb Spectronic 20 was used and the wavelength was read at 640 millimicrons.

7. Hydrolysis of Polysaccharide

Samples of the water-soluble and water-insoluble purified pentosan preparations from each flour source, together with samples of all the fractions obtained therefrom were hydrolyzed. Approximately 200-400 ug of each sample were hydrolyzed with 1 N sulfuric acid for 1½ hours on a vigorously boiling water bath. The hydrolysates

were neutralized by passing through small columns, containing an anion-exchange resin, Dowex 1-X10 (acetate form). The samples were evaporated to dryness, dissolved in 2 ml of the starting buffer (0.125 M boric acid pH 7.0) and then frozen.

8. Analyses of Component Sugars

The ratios of component sugars in the various pentosan fractions were determined on a Technicon Auto Sugar Analyzer according to the procedure of Kesler (49). The column used was 6 mm in diameter and 75 cm in length. The Technicon anion resin used was designated as Chromobeads, Type S 8% cross-linked (Technicon International of Canada Ltd.). The column contained a resin bed of 65 cm. The 2 borate buffers used to form the gradient for elution were: (1) 0.125 M boric acid pH 7.0, and (2) 0.6 M boric acid pH 10.0. A flow rate of 0.38 ml per min was used. Standard curves of xylose, arabinose and glucose were made by using different amounts and plotting the peak areas vs amount sugar.

IV RESULTS AND DISCUSSION

While wheat flours have received the predominant attention in regard to pentosan investigations, relatively little is known about the pentosans in durum and rye. No information is available on Triticale pentosans.

In the present investigation it was shown that a particular line of Triticale (6A190) did contain both water-soluble and water-insoluble pentosans. In general, it did not appear that the pentosan forming enzymes of the interspecific hybrid, Triticale, were simply derived as they existed in one or both of its parents. To interpret many of the findings in this study it is necessary to consider not only interaction between the factors coding for pentosan forming enzymes, but also the rates and interactions of these enzymes as well.

It was found, however, in comparing Triticale, rye and durum pentosans that specific Triticale pentosan components were similar to either or both of its parents, and in some cases appeared to be intermediate in nature.

1. Yield and Protein Content of Purified Pentosan Preparations

Table 1 shows the four flours used and their protein content according to the macro Kjeldahl procedure and based on a zero percent moisture content. The yield and protein content of the purified water-soluble and water-

Table 1. Protein content of flours milled from cereal varieties grown in 1967.

Cereal variety or line	Protein* %
Hard Red Spring (HRS) wheat (Manitou)	16.2
Durum wheat (Stewart 63)	12.8
Spring Rye (Prolific)	9.1
Triticale (6A190)	13.4

* Protein contents are on a dry basis (N x 5.7)

insoluble pentosan preparations derived from these four flours are given in Table 2. All data are based on the average of two samples. It was not possible to obtain yields of rye and Triticale water-insoluble pentosans because of the large losses encountered during the physical separation of contaminating proteins. Triticale flour contained the least amount of water-soluble pentosans. Durum flour contained slightly more than Triticale, whereas rye flour contained 3 times as much soluble pentosan. HRS wheat flour contained more soluble pentosan than either Triticale or durum, but considerably less than rye. The Triticale soluble pentosans contained a slightly

Table 2. Pentosan yields and protein contents of purified water-soluble and water-insoluble preparations from indicated flours.

	Water-soluble Pentosans		Water-insoluble Pentosans	
Pentosan source	Yield* %	Protein %	Yield %	Protein %
Manitou	0.38	3.0	0.19	6.6
Stewart 63	0.31	2.5	0.17	14.0
Prolific	0.92	2.8	....	11.7
6A190	0.30	3.3	....	6.9

* As % of original flour

higher protein content than either the rye, durum or HRS pentosans. The HRS flour contained more water-insoluble pentosan than the durum flour. The protein contents of the Triticale and rye water-insoluble pentosans varied considerably as a result of the physical separation technique employed.

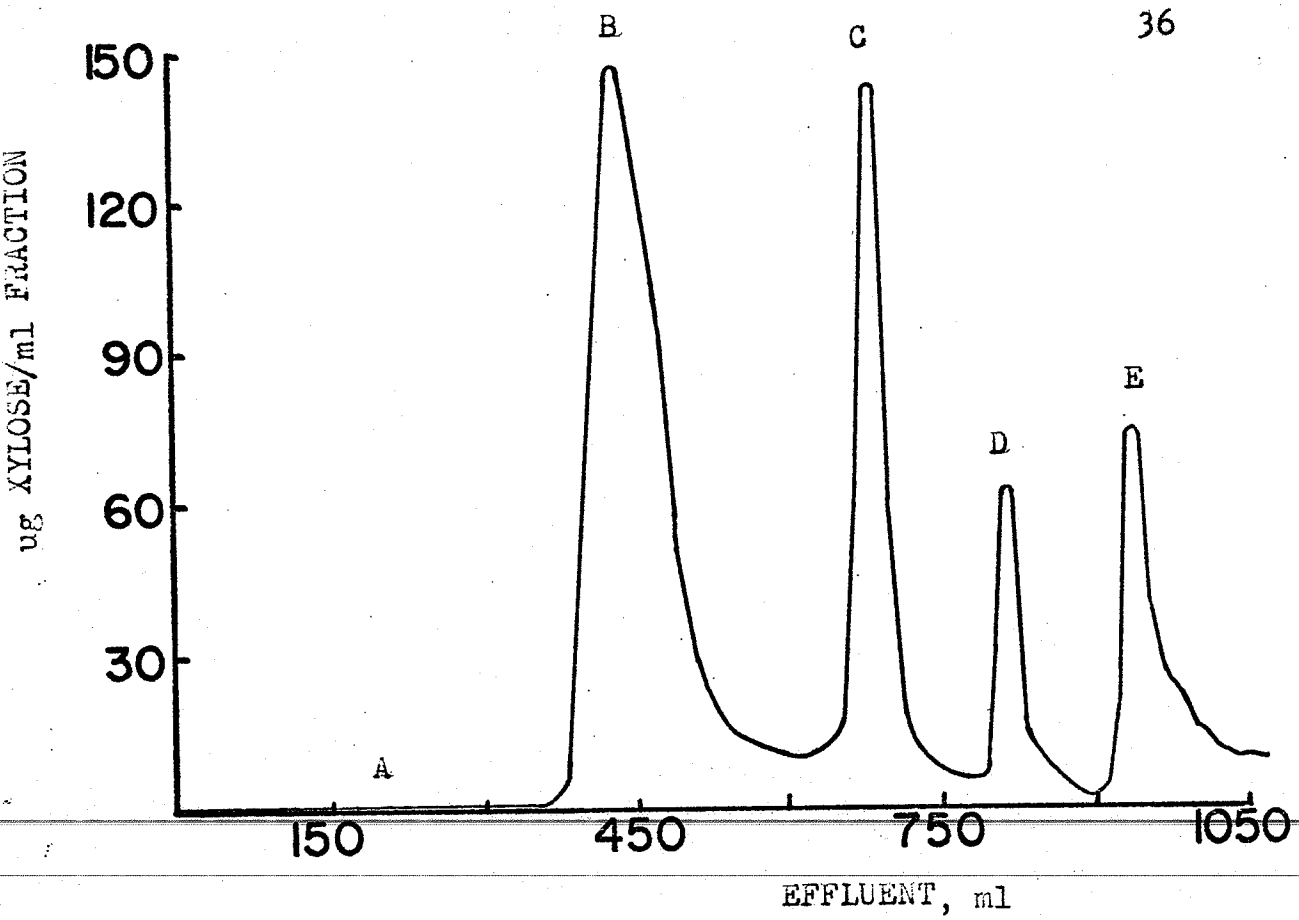
On a comparative basis, the yield data agree with other reports (30,50) in that HRS flours contain a slightly higher pentosan content than do durum flours, and secondly that rye flours contain approximately twice as much soluble pentosan as do HRS flours (29). The purified water-soluble pentosans from a HRS cv Manitou flour in the present study yielded 0.38% and contained 3.0% protein, as compared to purified water-soluble pentosans from another HRS cv Marquis flour which yielded 0.55% and contained 22.6% protein (30).

2. Distribution of Carbohydrates and Proteins in Fractionated Pentosans

The solubilized neutral pentosan preparations were concentrated and then fractionated on DEAE-cellulose ion-exchange columns (borate form). Four fractions were obtained in each case, except for the durum water-soluble pentosans, in which case five fractions were obtained. Typical results of column fractionation of both the water-soluble and water-insoluble pentosans from HRS, durum, rye and Triticale flours are given in Fig.'s 2 to 9.

Fig. 2. Fractionation on DEAE-cellulose columns of water-soluble pentosans (30 mg) from HRS wheat flour. Elution patterns of 15 ml fractions; A, distilled water; B, 0.0015 M $K_2B_4O_7$; C, 0.015 M $K_2B_4O_7$; D, 0.250 M $K_2B_4O_7$; and E, 0.3 N NaOH.

Fig. 3. Fractionation on DEAE-cellulose columns of water-insoluble pentosans (30 mg) from HRS wheat flour. Elution patterns of 15 ml fractions; A, distilled water; B, 0.0050 M $K_2B_4O_7$; C, 0.025 M $K_2B_4O_7$; D, 0.250 M $K_2B_4O_7$; and E, 0.4 N NaOH.


150
120
90
60
30
ug XYLOSE/ml FRACTION
A
B
C
D
E
150
450
750
1050
EFFLUENT, ml

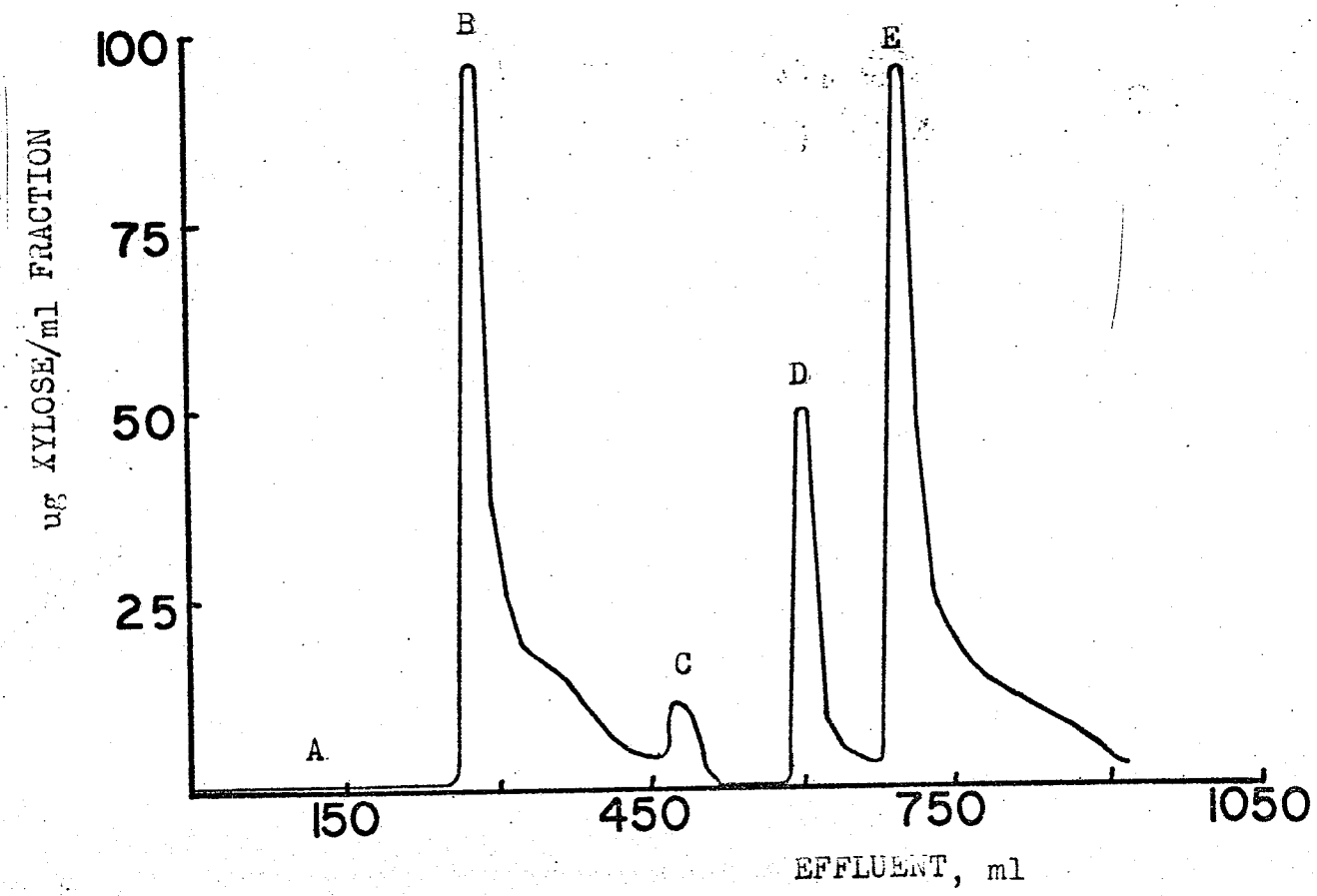
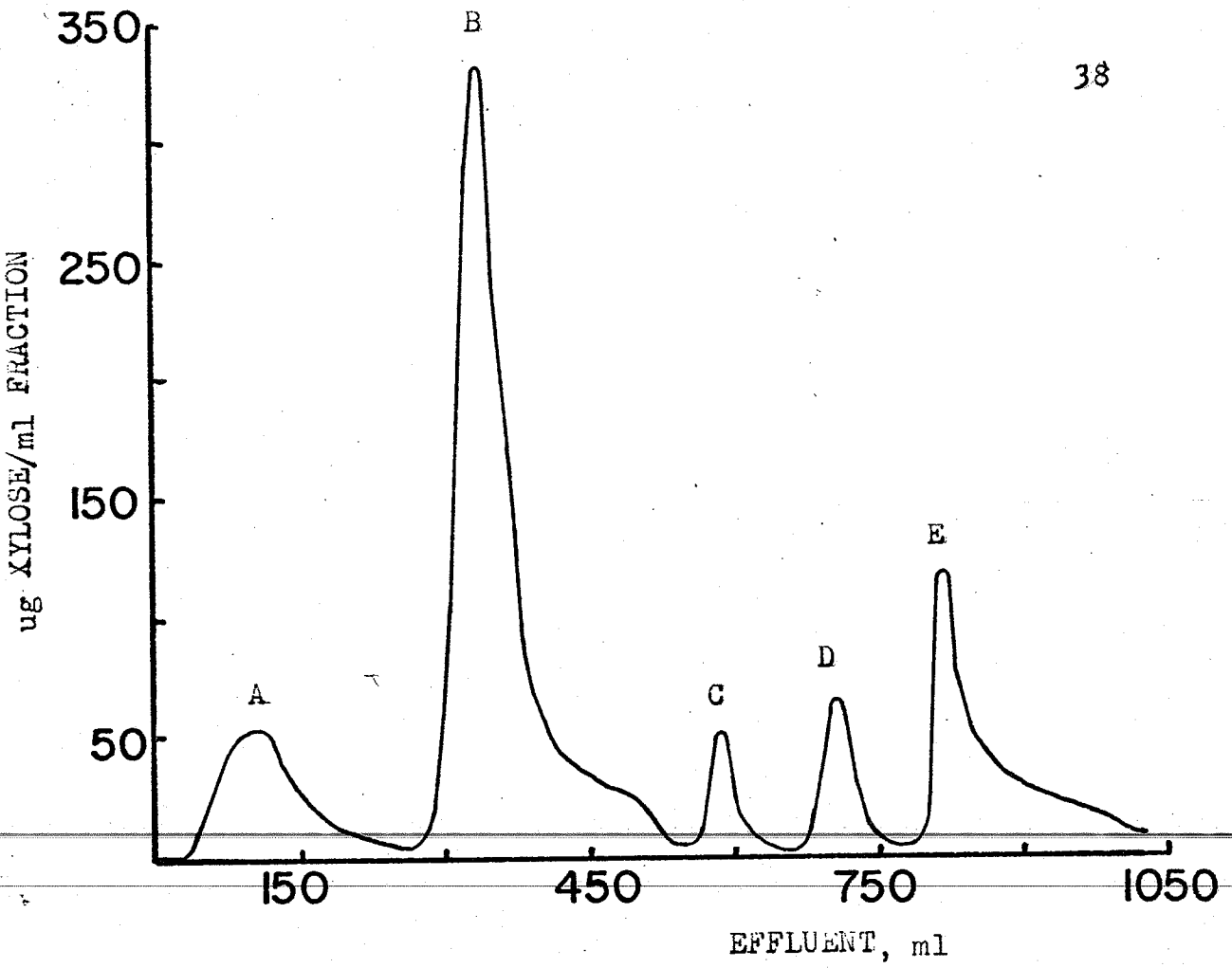

100
75
50
25
ug XYLOSE/ml FRACTION
A
B
C
D
E
150
450
750
1050
EFFLUENT, ml

Fig. 4. Fractionation on DEAE-cellulose columns of water-soluble pentosans (35 mg) from durum wheat flour. Elution patterns of 15 ml fractions; A, distilled water; B, 0.0025 M $K_2B_4O_7$; C, 0.025 M $K_2B_4O_7$; D, 0.250 M $K_2B_4O_7$; and E, 0.4 N NaOH.

Fig. 5. Fractionation on DEAE-cellulose columns of water-insoluble pentosans (30 mg) from durum wheat flour. Elution patterns of 15 ml fractions; A, distilled water; B, 0.0050 M $K_2B_4O_7$; C, 0.025 M $K_2B_4O_7$; D, 0.250 M $K_2B_4O_7$; and E, 0.4 N NaOH.


350
250
150
50
ug XYLOSE/ml FRACTION
A
B
C
D
E
150
450
750
1050
EFFLUENT, ml

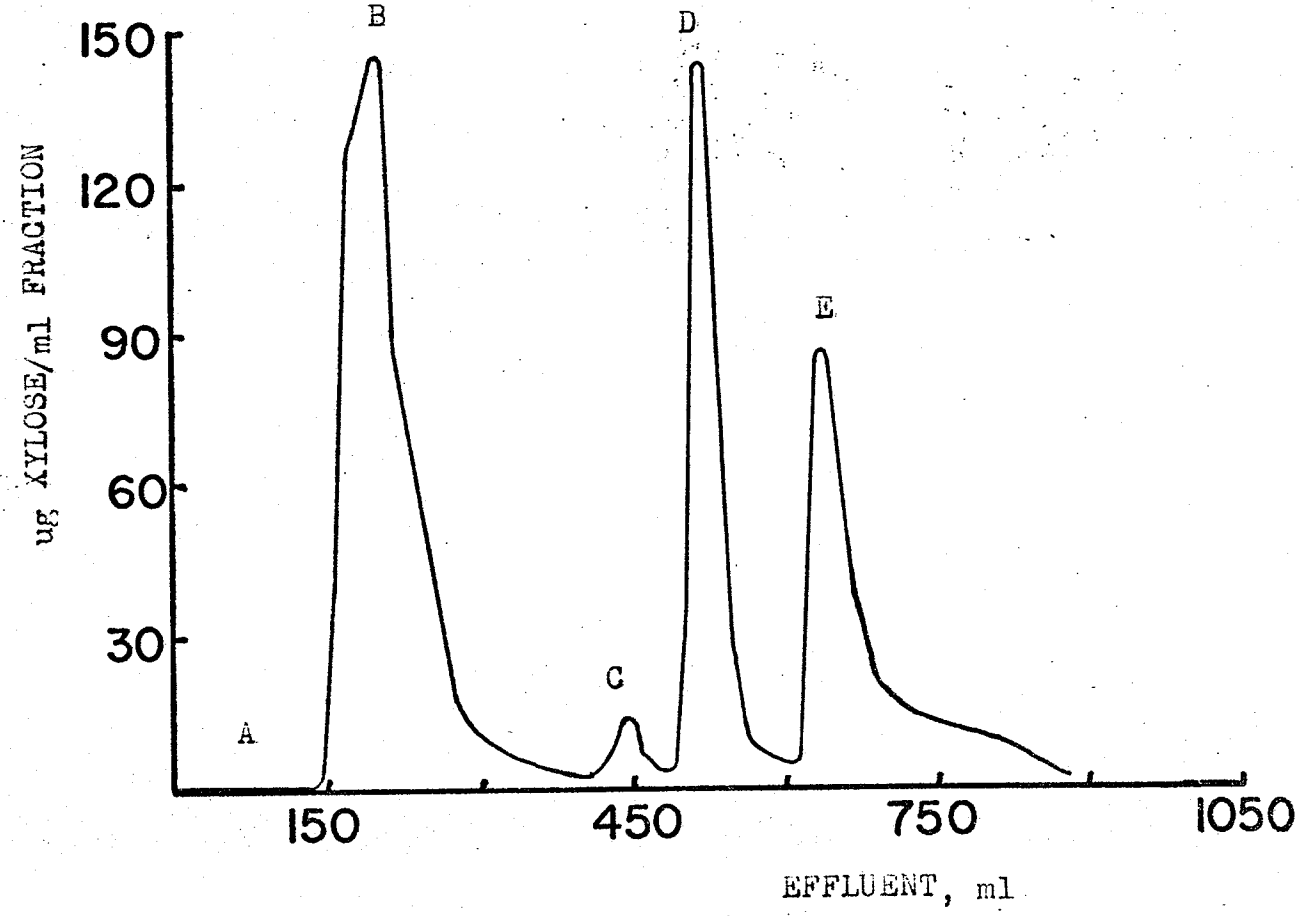
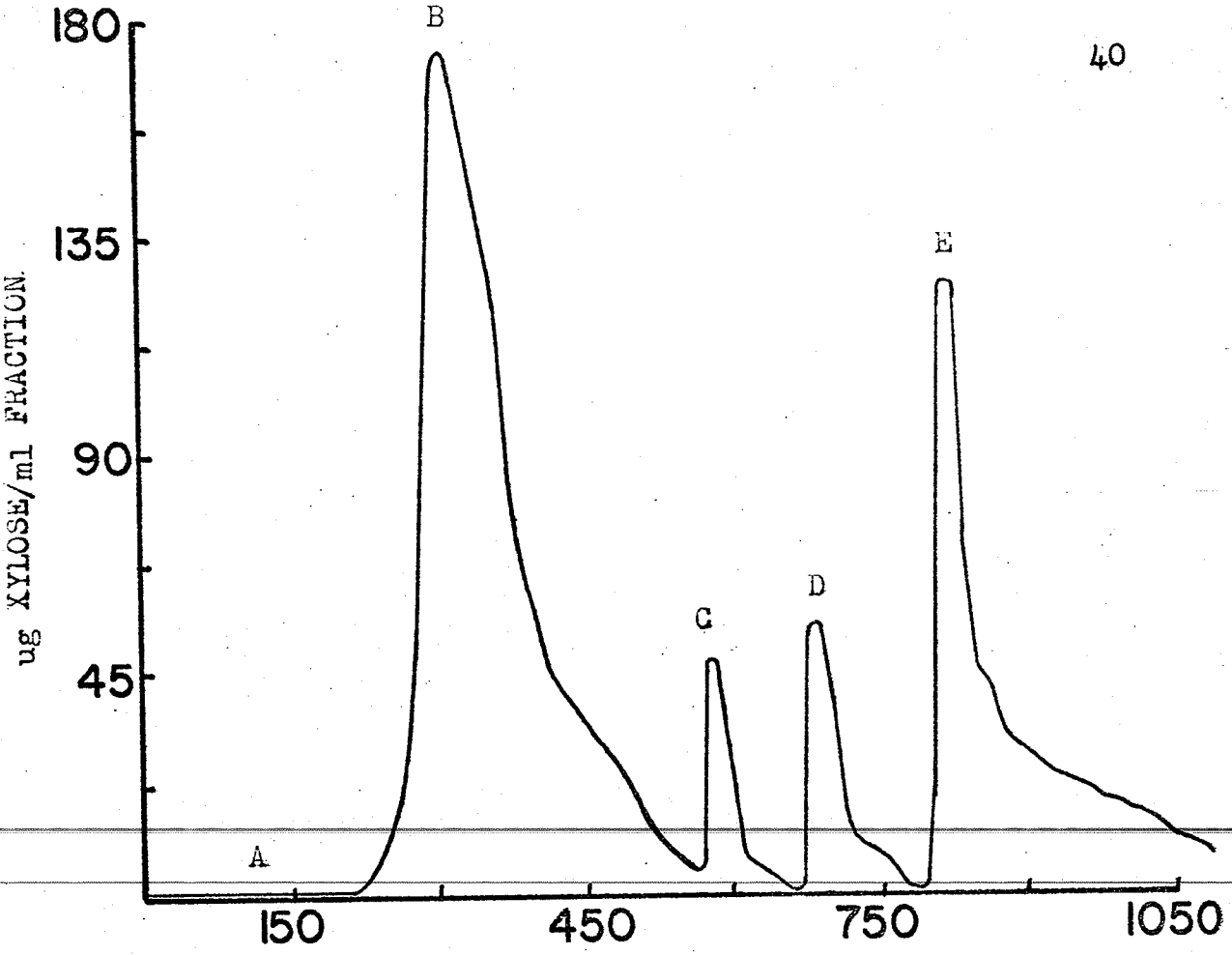

150
120
90
60
30
ug XYLOSE/ml FRACTION
A
B
C
D
E
150
450
750
1050
EFFLUENT, ml

Fig. 6. Fractionation on DEAE-cellulose columns of water-soluble pentosans (35 mg) from rye flour. Elution patterns of 15 ml fractions; A, distilled water; B, 0.0025 M $K_2B_4O_7$; C, 0.025 M $K_2B_4O_7$; D, 0.250 M $K_2B_4O_7$; and E, 0.4 N NaOH.

Fig. 7. Fractionation on DEAE-cellulose columns of water-insoluble pentosans (20 mg) from rye flour. Elution patterns of 15 ml fractions; A, distilled water; B, 0.0050 M $K_2B_4O_7$; C, 0.050 M $K_2B_4O_7$; D, 0.500 M $K_2B_4O_7$; and E, 0.4 N NaOH.


180
135
90
45
ug XYLOSE/ml FRACTION
A
B
C
D
E
150
450
750
1050
EFFLUENT, ml

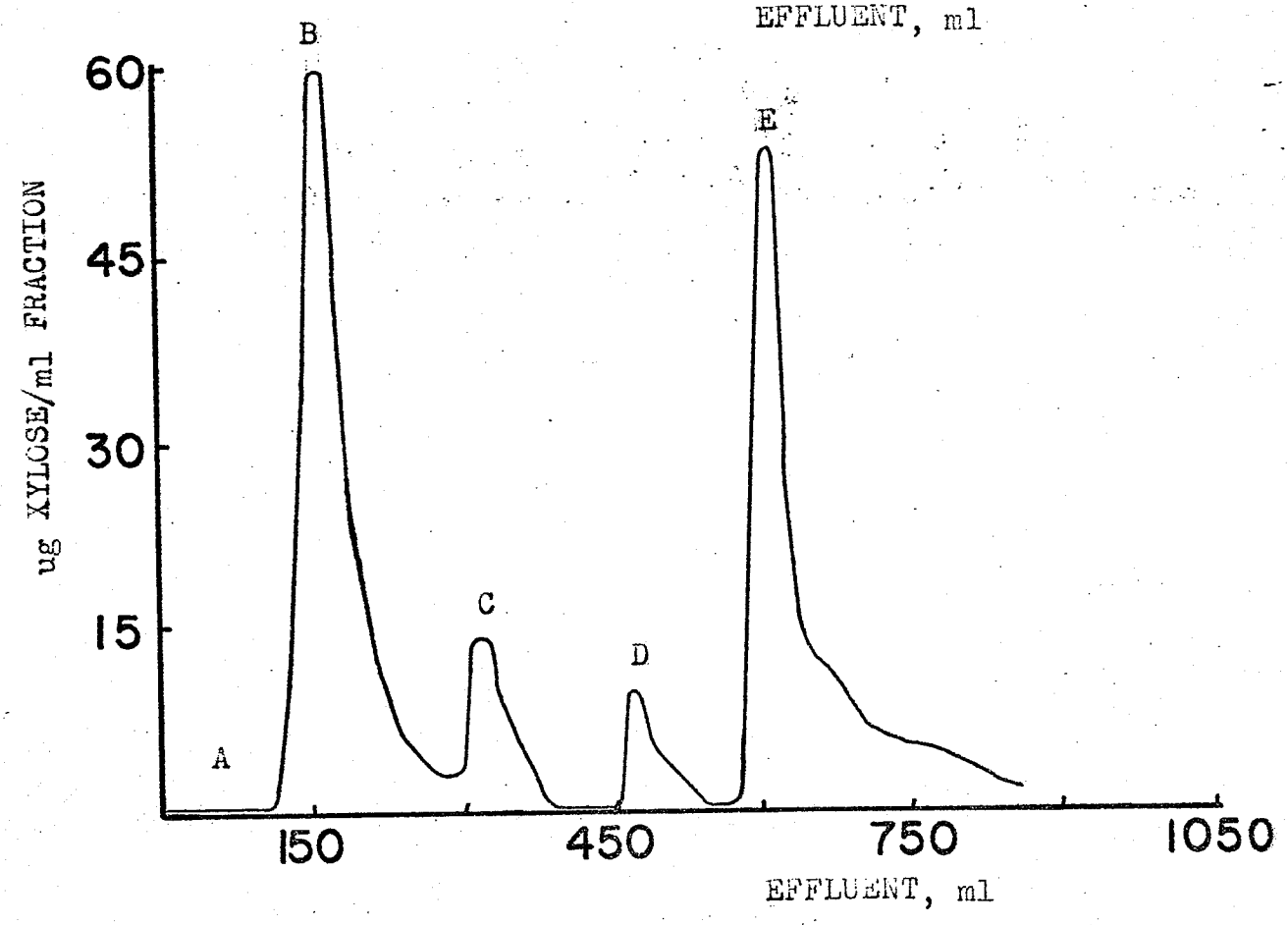
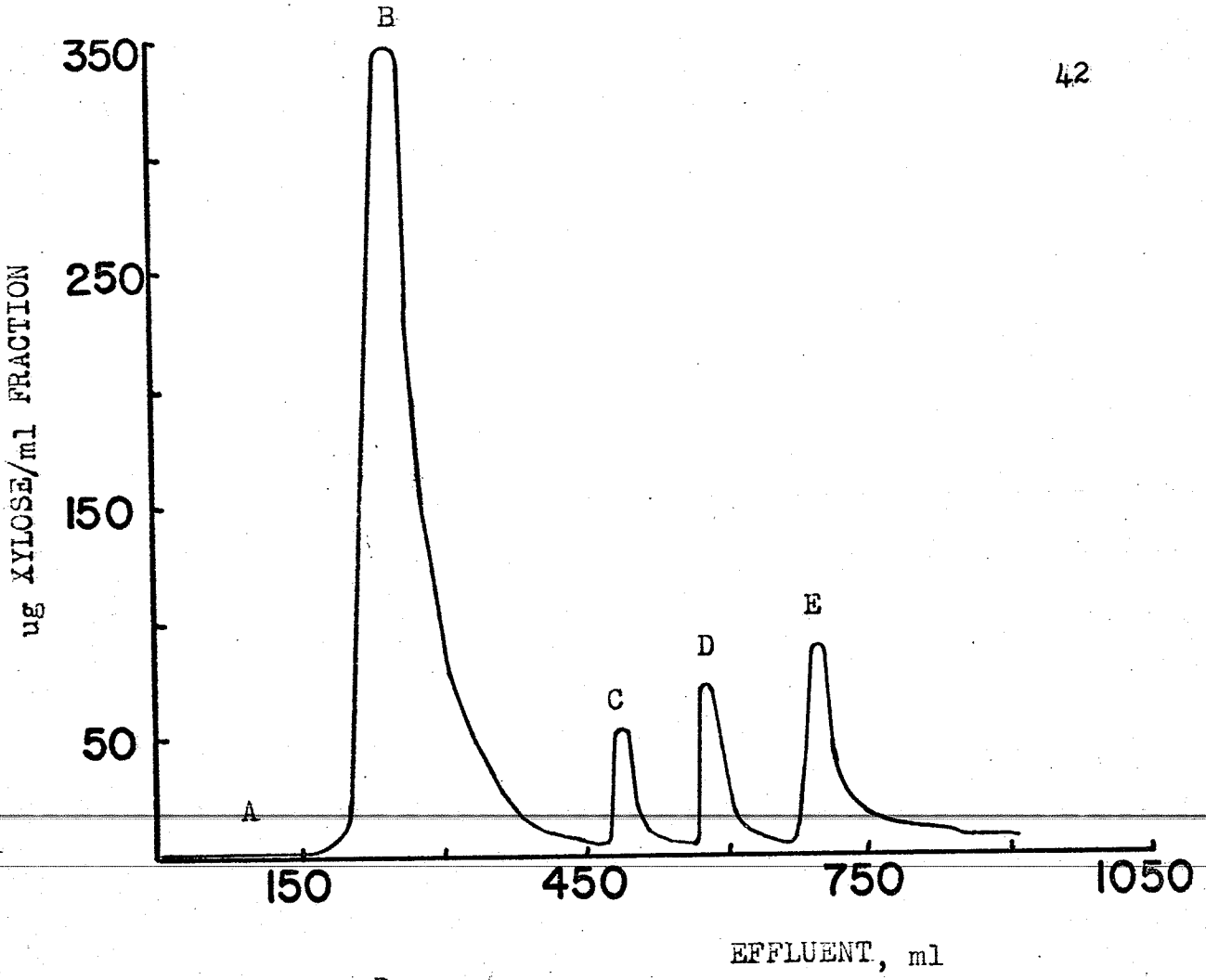
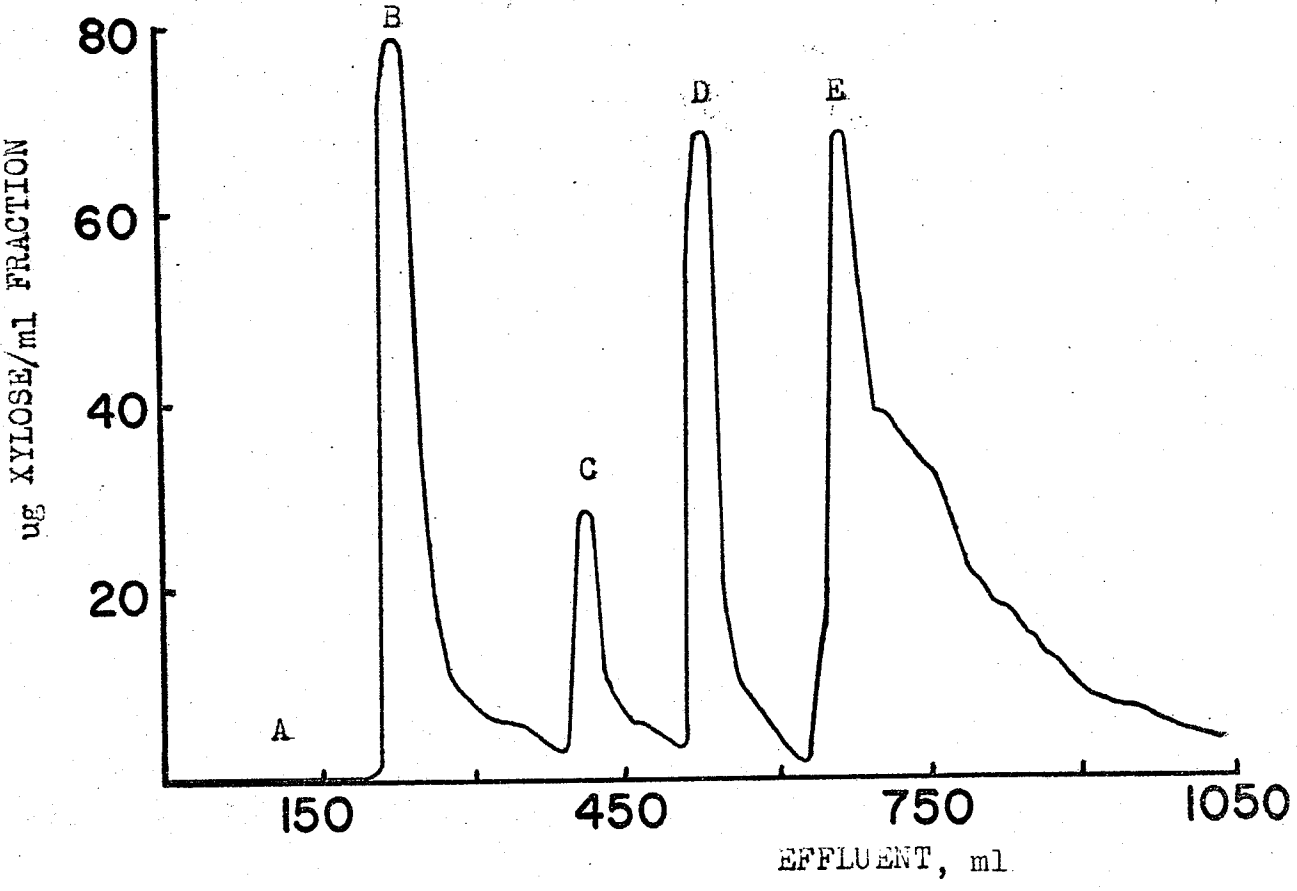

60
45
30
15
ug XYLOSE/ml FRACTION
A
B
C
D
E
150
450
750
1050
EFFLUENT, ml

Fig. 8. Fractionation on DEAE-cellulose columns of water-soluble pentosans (40 mg) from Triticale flour. Elution patterns of 15 ml fractions; A, distilled water; B, 0.0025 M $K_2B_4O_7$; C, 0.025 M $K_2B_4O_7$; D, 0.250 M $K_2B_4O_7$; and E, 0.4 N NaOH.

Fig. 9. Fractionation on DEAE-cellulose columns of water-insoluble pentosans (30 mg) from Triticale flour. Elution patterns of 15 ml fractions; A, distilled water; B, 0.0065 M $K_2B_4O_7$; C, 0.050 M $K_2B_4O_7$; D, 0.500 M $K_2B_4O_7$; and E, 0.4 N NaOH.


B
350
250
150
50
ug XYLOSE/ml FRACTION
A
C
D
E
150
450
750
1050
EFFLUENT, ml


B
80
60
40
20
ug XYLOSE/ml FRACTION
A
C
D
E
150
450
750
1050
EFFLUENT, ml

Changes in eluant composition or concentration were made after the eluate following an elution peak contained little or no carbohydrate. Generally, small differences in concentration of the initial potassium borate buffer resulted in little variation in the amount of total carbohydrate eluted. In the case of HRS soluble pentosans, however, when the molarity is increased from 0.0015 M to 0.0025 M, the total carbohydrate in the B fraction is increased by about 15%. Fraction C on the other hand is decreased proportionately.

Recoveries of water-soluble pentosans applied to the column averaged 93%, 92%, 97% and 97%, whereas the water-insoluble pentosans averaged 90%, 73%, 43% and 59% for HRS, durum, rye and Triticale respectively. In all cases but one, all of the material applied to the column formed a complex with borate. The water-insoluble pentosans appeared to form a more stable borate complex than their water-soluble counterparts. This was indicated by the lower recovery rates obtained upon fractionation. A relatively high percent of rye and Triticale water-insoluble pentosans was not removed even by prolonged elution with 0.4 N sodium hydroxide.

Distribution of total carbohydrate contents in the water-soluble pentosan fractions from the four flours (average of 2 samples) are shown in Table 3. The duplicate samples, while generally close, varied up to 15%. Durum

Table 3. Distribution of carbohydrates in water-soluble pentosan fractions eluted from DEAE-cellulose columns.

Pentosan source	Carbohydrate* in fraction A %	B %	C %	D %	E %
Manitou	0	55.4	22.5	7.6	19.7
Stewart 63	12.1	57.0	3.5	4.4	22.5
Prolific	0	65.8	3.3	5.7	25.1
6A190	0	77.6	3.5	6.8	12.0

* As % of total eluted from column

soluble pentosans were the only ones that yielded a water-eluted fraction. Fraction B was the largest in each case while fractions C and D generally represented the smallest fractions. HRS fraction C was much larger than the others, and is partially due, at any rate to the fact that a weaker elution pattern was used. Fractions C and D of Triticale were not too dissimilar to those of its parents. The most significant finding, however, was an increase in fraction B and a decrease in fraction E in going from durum and rye to Triticale. These differences of the major pentosan fractions in Triticale from those of its parents indicates that these pentosan fractions are not directly inherited from its parents.

Table 4 shows the distribution of the total carbohydrate contents in the water-insoluble pentosan fractions from the four flours (average of 2 samples) used. No A fraction was obtained from any of the purified water-insoluble pentosan preparations. While fraction B for both durum and rye was the largest fraction, for Triticale fraction E was the largest. Fraction C was the smallest fraction in each case. Triticale C fraction was similar to that found in rye. Triticale D fraction was intermediate to that of its rye and durum parents. For the water-insoluble pentosans, in contrast to the water-soluble ones, a decrease in fraction B with an accompanying increase in fraction E was obtained in going from durum and rye to Triticale. As was the case for the soluble Triticale

Table 4. Distribution of carbohydrates in water-insoluble pentosan fractions.

Pentosan source	Carbohydrate* in fraction A %	B %	C %	D %	E %
Manitou	0	40.3	2.8	13.3	43.4
Stewart 63	0	46.6	2.2	25.7	26.6
Prolific	0	38.2	8.1	15.7	36.9
6A190	0	29.1	7.9	18.7	44.3

* As % of total eluted from column

pentosan fractions, the major water-insoluble Triticale fractions appeared to contain different quantities of total carbohydrates than those of its parent species.

There appears to be a general trend of fractionation for both soluble and insoluble pentosans. The first fraction obtained is generally the largest one. It is followed by 2 fractions which are relatively small. The final fraction in a few cases is larger than the initial one, however, generally it is smaller. This same general pattern is in keeping with the reports of others (30,44).

In comparing the major water-soluble and water-insoluble pentosan fractions, the greatest variation occurs within Triticale B and E fractions. It is noteworthy that Triticale water-soluble pentosans contain the largest B and smallest E fractions, while Triticale water-insoluble pentosans contain the smallest B and largest E fraction. It is apparent from this that Triticale component pentosans are not directly derived as they exist in either of the hybrid's parents.

The pentosan source exhibiting the least variation within the water-soluble and water-insoluble B and E pentosan fractions is durum. The water-insoluble durum pentosans, however, contain a D fraction which is much larger than its water-soluble D counterpart. The water-soluble durum pentosans are unique in that they contain an A fraction. In comparing the carbohydrate content in

the water-soluble and water-insoluble pentosan fractions, fairly large differences are evident in most cases.

Table 5 shows the protein distribution in the water-soluble pentosan fractions eluted from the DEAE-cellulose columns. The percent protein in each fraction is based on the total xylose (anhydro) equivalent, as determined by the phenol-sulfuric acid carbohydrate test. This is in contrast with the macro and micro Kjeldahl protein determinations, which were based on the actual weight of the pentosan sample. On this account, the Lowry protein values were slightly higher than when protein was determined on an actual weight basis. The protein values are based on an average of 2 samples.

The B fractions contained very little protein, ranging from 1.7% for Triticale to 3.4% for durum. This is in agreement with other reports (25,30), indicating that the major fraction contains primarily carbohydrate material. Fractions C, D and E contain varying amounts of protein and are designated as glycoproteins. The D fractions contained the highest protein content in each case. Triticale D fraction was intermediate to the rye and durum D fractions, while its C and E fractions contained the highest protein content of all the C and E fractions.

The protein-carbohydrate ratio in the various fractions of Triticale and its parents varied considerably indicating that the glycoproteins were by no means similar.

Table 5. Distribution of proteins in water-soluble pentosan fractions eluted from DEAE-cellulose columns.

Pentosan source	Protein* in fraction A %	B %	C %	D %	E %
Manitou	0	2.7	5.4	15.0	5.1
Stewart 63	2.6	3.4	9.2	16.2	3.7
Prolific	0	2.2	9.0	10.4	7.3
6A190	0	1.7	12.2	14.8	9.7

* As % of total carbohydrate in fraction (Lowry protein values)

The question of whether or not defined glycoprotein molecules of fixed composition, size and structure exist in pentosans has by no means been settled. Nevertheless, it has been shown that pentosans contain protein which could not be removed, and therefore appears to be intimately associated with the pentosan molecule.

The protein distribution in the water-insoluble fractions is shown in Table 6. HRS and durum fractions B and C were essentially free of protein. Durum fractions E contained the largest amount of protein while the C fractions for the others contained the largest protein content. Triticale and rye B fractions contained minute quantities of protein. With the exception of fraction E, the protein contents of fractions B, C and D of Triticale were intermediate to those of its rye and durum parents. In general it appeared that the last 2 fractions, D and E, contained most of the protein. It is not known whether this protein is chemically bound to the pentosan, but it is likely that fractions D and E are glycoproteins, such as have been found in water-soluble pentosans. The protein was not monitored photometrically as it was eluted from the column, and therefore it is unknown whether the carbohydrate and protein peaks coincided.

3. Analyses of Hydrolyzed Pentosan Preparations and Fractions

The purified pentosan preparations, after hydrolysis,

Table 6. Distribution of proteins in water-insoluble pentosan fractions.

Pentosan source	A %	B %	C %	D %	E %
Manitou	0	0.7	0	12.5	7.9
Stewart 63	0	0.6	...[a]	10.0	18.7
Prolific	0	2.6	10.6	22.3	17.8
6A190	0	1.0	4.8	11.7	10.5

* As % of total carbohydrate in fraction

...[a] Material insufficient for testing

were qualitatively and quantitatively analyzed using a Technicon Auto Sugar Analyzer. All pentosans were found to contain the pentose sugars xylose and arabinose. Small amounts of glucose were found in the soluble and insoluble rye pentosans and was probably due to incomplete starch digestion by the alpha-amylase treatment. Rye pentosans have been reported to contain considerably higher molecular weight components than hard, soft or durum wheat pentosans (33), and therefore it is possible that the starch may become more entrapped within the pentosan polymer. Small amounts of glucose also appeared in a few of the fractions obtained by column chromatography, and must have come from DEAE-cellulose fragments eluted off the column. No galactose was found in the water-soluble pentosans which is probably due to the purification procedure used (12,22).

From most investigations to date, it does not appear that glucose forms a distinct part of the pentosan molecule, and therefore in the present study, in the few instances where glucose appeared, it was not included in the sugar ratios. The sugar ratios indicate the degree of branching in the pentosan molecule. In recent years, various attempts have been made to separate the heterogeneous pentosan molecule into homogeneous fractions, and the most commonly used method of characterizing the fractions obtained has been by determing the degree of branch-

ing. In the present study the criteria used to determine whether or not the pentosans of Triticale are derived directly from its parents is based on similarities of arabinose-xylose ratios. Differences in A/X ratios of similar fractions between Triticale and either of its parents precludes simple inheritance. It is assumed that each of the fractions obtained represents a homogeneous pentosan.

The ratio of component sugars in the unfractionated or purified water-soluble and water-insoluble pentosan preparations are given in Table 7. There were relatively small arabinose-xylose (A/X) ratio differences between the pentosans from the 4 flours. This indicates that the pentosans from these cereal flours are not very dissimilar in terms of pentose sugar composition. For the water-soluble pentosans, the A/X ratio extended from 1:1.2 for Triticale to 1:1.6 for HRS, while for the water-insoluble pentosans it was from 1:1.2 for Triticale to 1:1.5 for HRS. Durum pentosans were found, as has been reported previously (33), to be slightly higher branched than those from HRS.

With the exception of Triticale, the water-insoluble pentosans were more highly branched than the corresponding water-soluble pentosans. Triticale soluble and insoluble pentosans had the same A/X ratio, indicating that both contained the same degree of branching. Both rye and durum

Table 7. Pentose sugar composition of hydrolyzed water-soluble and water-insoluble purified pentosan preparations.

	Water-soluble pentosans	Water-insoluble pentosans
Pentosan source	Ratio,* Arabinose:Xylose	Ratio, Arabinose:Xylose
Manitou	1 : 1.6	1 : 1.5
Stewart 63	1 : 1.4	1 : 1.3
Prolific	1 : 1.5	1 : 1.3
6A190	1 : 1.2	1 : 1.2

* Ratios are based on anhydro sugar present

soluble and insoluble pentosans were less highly branched, or more linear than those from Triticale. This observation further indicated that the pentosans in the hybrid are not inherited directly from its parents. Both rye soluble and insoluble pentosans contained a pentosan component which had a very high A/X ratio. It could be interpreted from this that rye contains a genetic factor which could account for the higher degree of branching in Triticale pentosans.

The fractions obtained using the DEAE-cellulose column chromatography procedure were also analyzed for their sugar composition. Different arabinose-xylose percentages were found to occur in the fractions obtained from each purified pentosan preparation. Except for unexpected or unusually high or low A/X ratios, in which case at least 2 sugar determinations were made, A/X ratios are based on one determination. Duplicate runs generally varied by less than 5%.

In all cases, fractions were obtained which were both more highly and less highly branched than the unfractionated purified pentosan preparations. This indicated, that HRS, durum, rye and Triticale water-soluble and water-insoluble pentosans consist of more than one molecular species. The first and the last fractions were generally less branched than the two intermediate fractions. This is in agreement with another report in which the first

fraction from HRS, durum and rye water-soluble pentosans was found to be less highly branched than the second fraction (33).

The A/X ratio of the soluble and insoluble Triticale pentosan fractions are given in Table 8. The Triticale fractions are unique in that the soluble and insoluble fractions have the same sugar ratio. In addition, the fractions have a relatively small range in A/X ratios. Triticale pentosans, therefore, appear to be less heterogeneous than those from rye and durum. Triticale did not contain any fraction that had a different A/X ratio than existed within the fractions of its parents. When comparing similar fractions, however, it is apparent that the A/X ratios of Triticale pentosan fractions are different than those of either of its parents. This indicates, therefore, that some sort of genetic modification must have occured insofar as Triticale pentosans are concerned.

Fractions D and E were the highest and lowest branched fractions respectively. The sugar ratios of fractions C, D and E for Triticale soluble pentosans were intermediate to those of the corresponding fractions. Fractions B and C were identical in their degree of branching, and could both be the same pentosan component, or differ in their molecular size or shape. Various physical tests, however, would be required to confirm this.

Table 9 shows the arabinose-xylose ratios of the

Table 8. Analyses of Triticale water-soluble and water-insoluble pentosan fractions.

Fraction		Water-soluble pentosans Ratio, Arabinose:Xylose	Water-insoluble pentosans Ratio, Arabinose:Xylose
6A190	B	1 : 1.2	1 : 1.2
	C	1 : 1.2	1 : 1.2
	D	1 : 1.0	1 : 1.0
	E	1 : 1.4	1 : 1.4

Table 9. Analyses of Durum and Rye water-soluble and water-insoluble pentosan fractions.

Fractions		Water-soluble pentosans Ratio, Arabinose:Xylose	Water-insoluble pentosans Ratio, Arabinose:Xylose
Stewart 63	A	1 : 2.1	
	B	1 : 1.4	1 : 1.3
	C	1 : 1.4	...[a]
	D	1 : 1.2	1 : 1.2
	E	1 : 1.5	1 : 1.5
Prolific	B	1 : 1.5	1 : 1.4
	C	1 : 1.0	1 : 1.0
	D	1 : 0.7	1 : 0.8
	E	1 : 2.5	1 : 1.3

...[a] Material insufficient for testing

durum and rye pentosan fractions. The durum and rye pentosans were quite different in terms of branching. Rye contained both soluble and insoluble pentosan fractions which were much more highly branched than any of the durum fractions. Fraction D in all cases contained the highest A/X ratio. Durum and rye water-soluble pentosans each contained a fraction which was very linear. The durum linear fraction was eluted with water whereas the rye linear fraction was eluted with base. This indicates that these 2 pentosan molecular species must be quite dissimilar.

Durum water-soluble pentosans were unique in that a fraction A was obtained. This fraction was not inherited by Triticale. Fraction A had a very low A/X ratio, indicating that the molecules contained a low degree of branching. The durum water-insoluble pentosan fractions had a sugar ratio which was not too different from the water-soluble pentosan fraction, and were therefore similar in this respect to the Triticale pentosans.

Rye soluble pentosans contained a fraction, D, which was the most highly branched, and a fraction, E, which was the most linear of any fraction. The water-insoluble pentosan fraction D of rye was the most highly branched of the water-insoluble pentosan fractions. It appears that rye pentosan fraction D is coded by a genetic factor which is capable of producing a very highly branched pen-

tosan molecule. It is postulated that this genetic factor might account for the higher degree of branching which occurs in Triticale pentosans.

The Triticale insoluble pentosan fractions had sugar ratios which were intermediate to those of its parents insoluble pentosan fractions. The Triticale soluble pentosan fractions were not intermediate but had sugar ratios which were more like those of its durum parent.

The arabinose-xylose ratios of the HRS soluble and insoluble pentosan fractions are shown in Table 10. The pentosans from HRS wheat flour were more linear than those from the other flours. The procedure used to isolate the water-soluble pentosans yielded a purified product which was considerably more linear than the purified pentosans obtained by some other investigators. However, the A/X ratio of pentosans varies according to the isolation procedure used, and therefore it is virtually impossible to compare data on pentosans without knowing the precise procedures employed. The particular isolation procedure used in this investigation for obtaining water-soluble pentosans was desirable from the standpoint that protein contamination was reduced to a minimum. Water-insoluble pentosans of rye and Triticale were exceedingly difficult to obtain, since no satisfactory isolation procedures have yet been devised.

The water-insoluble HRS pentosans had a slightly

Table 10. Analyses of HRS water-soluble and water-insoluble pentosan fractions.

Fraction		Water-soluble pentosans Ratio, Arabinose:Xylose	Water-insoluble pentosans Ratio, Arabinose:Xylose
Manitou	B	1 : 1.8	1 : 1.5
	C	1 : 1.3	1 : 1.0
	D	1 : 1.4	1 : 1.2
	E	1 : 1.6	1 : 1.7

higher A/X ratio than the soluble HRS pentosans. It was found, as has been reported, that durum pentosan fractions were slightly higher branched than the corresponding HRS fractions (33).

In plants, carbohydrate substances are synthesized by specific enzymes which are directly coded for by a gene or any number of genes on specific chromosomes. In attempting to elucidate the mode of inheritance of pentosans in the hybrid, Triticale, two important considerations are necessary. These are first, the interaction between the genetic determinants coding for the pentosan forming enzymes, and second, the rates and interactions of these enzymes.

On a quantitative basis, the water-soluble pentosan content of Triticale is similar to that of its durum parent. Thus either A or B or both genomes appear to be responsible for the production of enzymes which regulate the quantity of pentosan produced in Triticale.

While it cannot be unequivocally stated that the pentosan fractions obtained from each purified pentosan preparation consisted entirely of one molecular species, the fractions did differ in their protein and pentose sugar content. On the assumption that these fractions are reproducible within a cereal species, and are characteristic of that species, it is possible to speculate inheritance of these distinct fractions in Triticale. Triticale water-

soluble fraction B contained considerably more carbohydrate than either parent, indicating that either a high level of pentosan synthesizing enzymes were present, or that the reaction rates of the pentosan forming enzymes were very high. Triticale water-soluble fraction E, conversely, contained only about half the quantity of carbohydrate as that of its parents, thereby indicating a low enzyme level or slower reaction rates. Triticale water-insoluble fraction B was smaller, and fraction E larger than the corresponding fractions of its parents. It is apparent from this that there exists differences in regulation of pentosan forming enzymes between hybrid and parents. The protein content in the glycoprotein fractions fluctuated considerably and therefore it is exceedingly difficult to formulate a meaningful protein pattern of inheritance. It was significant, however, that fraction D in all cases except one represented the largest glycoprotein fraction. Both soluble and insoluble Triticale D pentosan fractions had protein contents which were intermediate between those of its parent species, durum wheat and rye.

Triticale pentosans had the highest degree of branching of the four cereals examined. It is postulated that the R genome of rye contributed to an increase in the degree of branching of Triticale pentosans by effecting an increase in the level and/or activity of branching enzyme(s). This is based on the fact that rye pentosans

contained fractions which were very highly branched.

Of the cereals examined, Triticale pentosans were the least heterogeneous. The respective water-soluble and water-insoluble pentosan fractions exhibited the same degree of branching. The variation in degree of branching between fractions was relatively small, and is quite possibly due to interactions between enzymes which are responsible for linearity and branching.

V SUMMARY

1. The pentosans of one line of Triticale (6A190), its parental species (T. turgidum L., ssp durum, cv. Stewart 63 and Secale cereale, cv. Prolific), and one variety of T. aestivum L. em Thell, ssp. aestivum, cv. Manitou were studied.

2. Triticale, an interhybrid species, contained both water-soluble and water-insoluble pentosans. The quantity of water-soluble pentosans in Triticale flour, on a comparative basis, was essentially the same as that found in its durum parent. Rye flour contained approximately 3 times as much water-soluble pentosan as either durum or Triticale. All purified pentosan preparations contained some protein.

3. The pentosans from the four cereal flours could be fractionated, by using DEAE-cellulose (borate form) ion-exchange column chromatography, into fractions which differed in their protein and pentose sugar content. The soluble pentosans all yielded a large arabinoxylan fraction which contained very little protein, and 3 fractions (C,D,E) containing carbohydrate and protein, referred to as glycoproteins. Most of the protein in the fractionated water-insoluble

pentosans was located in the last 2 fractions, D and E.

4. The ratios of the two pentose component sugars, xylose and arabinose were determined in the purified pentosan preparations. The water-insoluble pentosans, with the exception of Triticale were slightly higher branched than the water-soluble pentosans. Triticale water-soluble and insoluble pentosans had the same degree of branching. HRS pentosans were the most linear of the four cereals examined. Durum pentosans were slightly higher branched than HRS pentosans. Triticale pentosans were the most highly branched.

5. The A/X ratios in the fractions were generally different, indicating heterogeneity of the starting pentosan material. Rye pentosan fractions showed the greatest range in A/X ratios, indicating that they were the most heterogeneous. The pentosan molecular species of the linear fractions in durum and rye appeared to be very different. The highly branched pentosan molecular species (fractions C and D), on the other hand, were much less diverse since they were eluted by a relatively small range in eluant concentration.

6. The pentosans in Triticale appear to be quite distinct from those of its parents. The evidence in this study indicates that the chemical make-up of the minor cereal component, pentosan, was altered when two very dis-

similar cereal species, rye and durum wheat were crossed to produce an entirely new inter-hybrid species, Triticale.

VI REFERENCES

1. Bechtel, W. G., Geddes, W. F., and Gilles, K. A. Carbohydrates In Wheat: Chemistry and technology, 1. Hlynka, ed., chap. 7. Monograph Series, Vol.III American Assoc. of Cereal Chemists. St. Paul, Minn. (1964).

2. Ritthausen, H. Verbindungen der Proteinstoffe mit Kupferoxid. J. Prakt. Chem. 5:215-225 (1872).

3. Haus, P., and Hill, T. G. An introduction to the chemistry of plant products. Vol. 1. Longmans, Green, and Co. London (1928).

4. Gudkow. Uber die "furfurolgebende Substanz" der Kleie. Z. Chem. (2) 6:360-366 (1870).

5. Stone, W. E., and Tollens, B. Versuche zur Aufsuchung von Materialien zur Arabinose gewinnung. Ann. 249:238-245 (1888).

6. Wroblewski, A. Uber die chemische Beschaffenheit der Diastase und uber das Vorkommen eines Arabans in den Diastase Preparaten. Ber. 30:2289-2295 (1897).

7. Geoffrey, R. Arabane des farines de froment. Bull. Soc. Chim. biol. 19:60-64 (1937).

8. Hoffman, W., and Gortner, R. A. The preparation and analysis of the various proteins of wheat flour with special reference to the globumin, albumin and proteose fraction. Cereal Chem. 4:221-229 (1927).

9. Freeman, M. E., and Gortner, R. A. The gums of the cereal grains. Cereal Chem. 9:506-518 (1932).

10. Holme, J. Characterization of soluble proteins and pentosans of cake flour. Cereal Chem. 39:132-144 (1962).

11. Kulp, K. Pentosans of wheat endosperm. Cereal Science Today. Vol. 13, No. 11 414-417 (1968).

12. Baker, J. C., Parker, H. K., and Mize, M. D. The pentosans of wheat flour. Cereal Chem. 20:267-280 (1943).

13. Pence, J. W., Elder, A. H., and Mecham, D. K. Preparation of wheat flour pentosans for use in reconstituted doughs. Cereal Chem. 27:60-66 (1950).

14. Vones, F., Podrazky, V., Simova, J., and Vesely, Z. Significance of wedge proteins of rye flour for dough properties. Cereal Chem. 41:456-464 (1964).

15. Preece, I. A., and Mackenzie, K. G. Nonstarchy polysaccharides of Cereal grains, II. Distribution of water-soluble gum like materials in cereals. J. Inst. Brewing 58:457-464 (1952).

16. Gilles, K. A., and Smith, F. The nature of the water-soluble polysaccharides of the flour of wheat. Cereal Chem. 33:29-37 (1956).

17. Perlin, A. S. Isolation and composition of the soluble pentosans of wheat flours. Cereal Chem. 28:370-381(1951).

18. Perlin, A. S. Structure of the soluble pentosans of wheat flours. Cereal Chem. 28:382-393 (1951).

19. Neukom, H., Providoli, L., Gremli, H., and Hui, P. A. Recent investigations on wheat flour pentosans. Cereal Chem. 44:238-244 (1967).

20. Montgomery, R., and Smith, F. The carbohydrates of Gramineae, VII. The constitution of a water soluble hemicellulose from the endosperm of wheat (Triticum vulgare). J. Amer. chem. Soc. 77:3325-3328 (1955).

21. Ewald, C. H., and Perlin, A. S. The arrangement of branching in an araboxylan from wheat flour. Canad. J. Chem. 37:1254-1259 (1959).

22. Preece, I. A., and Hobkirk, R. III. Higher molecular gums of common cereals. J. Inst. Brewing. 59:385-392 (1953).

23. Aspinall, G. O., and Ross, K. M. Degradation of two periodate-oxidized arabinoxylans. J. Chem. Soc. 1681-1686 (1963).

24. Pomeranz, Y. Cereal gums. Qualitas Plant. Materiae Veget. 8:158-194 (1961).

25. Kuendig, W., Neukom, H., and Deuel, H. Chromatographische Fraktionierung von wasserloslichen Weizenmehlpentosanen an DEAE-cellulose. Helv. Chim. Acta. 44:823-829 (1961).

26. Neukom, H., Kuendig, W., and Deuel, H. The soluble wheat flour pentosans. Cereal Sci. Today Vol. 7, No. 4. 112-125 (1962).

27. Montgomery, R., and Smith, F. The carbohydrates of Gramineae, V. The constitution of a hemicellulose of the endosperm of wheat (Triticum vulgare). J. Amer. chem. Soc. 77:2834-2837 (1955).

28. Kuendig, W. Untersuchungen uber die Zusammensetzung und Eigenschaften der Schleimstoffe des Weizenmehls. Prom. Nr. 3267 Zurich (1962).

29. Neukom, H. Final Report of Research conducted under Grant Authorized by Public Law 480 for the U.S.D.A. Swiss Fed. Inst. of Tech. Dept. Agric. Chem. Zurich Switzerland. June 1969.

30. Lin, F. M., and Pomeranz, Y. Characterization of water-soluble wheat flour pentosans. J. Food Sci. 33:599-606 (1968).

31. Neukom, H., Deuel, H., Heri, W. J., and Kuendig, W. Chromatographische Fraktionierung von Polysaccharides an Cellulose-Anionenaustauscher. Helv. Chim. Acta. 43:64-71 (1960).

32. Wrench, P. M. The role of wheat flour pentosans in baking, III. Enzymatic degradation of pentosan fractions. J. Sci. Food Agr. 16:51-57 (1965).

33. 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. 45:539-549 (1968).

34. MacMasters, M. M., and Hilbert, G. E. The composition of the "amylodextrin" fraction of wheat flour. Cereal Chem. 21:548-555 (1944).

35. Sandstedt, R. M., Jolitz, C. E., and Blish, M. J. Starch in relation to some baking properties of flour. Cereal Chem. 16:780-792 (1939).

36. Clendenning, K. A., and Wright, D. E. Separation of starch and gluten, V. Problems in wheat starch manufacture arising from flour pentosans. Canad. J. Res. F. 28:390-400 (1950).

37. Yamazaki, W. T. The concentration of a factor in soft wheat flours affecting cookie quality. Cereal Chem. 32:26-37 (1956).

38. Simpson, F. J. A survey of microorganisms for the production of enzymes that attack the pentosans of wheat flour. Can. J. Microbiology 1:131-139 (1954).

39. Kulp, K., and Bechtel, W. G. Effect of water-insoluble pentosan fraction of wheat endosperm on the quality of white bread. Cereal Chem. 40:493-504 (1963).

40. Perlin, A. S., and Suzuki, S. A note on the polysaccharides of wheat flour squeegee. Cereal Chem. 42:199-201 (1965).

41. Upton, E. M., and Hester, E. E. Nonstarchy polysaccharides and proteins of soft wheat flour tailings. Cereal Chem. 43:156-168 (1966).

42. Medcalf, D. G., and Gilles, K. A. Structural characterization of a pentosan from the water-insoluble portion of durum wheat endosperm. Cereal Chem. 45:550-556 (1968).

43. Abdel-Akher M., Hamilton, J. K., Montgomery, R., and Smith, F. A new procedure for the determination of the fine structure of polysaccharides. J. Amer. Chem. Soc. 74:4970-4971 (1952).

44. Cole, E. W. Isolation and chromatographic fractionation of hemicelluloses from wheat flour. Cereal Chem. 44:411-416 (1967).

45. Sollars, W. F., and Bowie, S. M. Effect of the subfractions of starch tailings on cookie diameter. Cereal Chem. 43:244-260 (1966).

46. Neukom, H., and Kuendig, W. Fractionation of neutral and acidic polysaccharides by ion-exchange column chromatography on diethylaminoethyl (DEAE)-cellulose. In Methods in carbohydrate chemistry. Vol. V. R. L. Whistler ed. Academic Press, New York (1965).

47. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356 (1956).

48. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein measurement with the Folin-phenol reagent. J. Biol. Chem. 193:265-275 (1951).

49. Kesler, R. B. Rapid quantitative anion-exchange chromatography of carbohydrates. Anal. Chem. 39:1416-1422 (1967).

50. Bains, G. S., and Irvine, G. N. The quality of Canadian amber durum wheat grades and the role of a pentosan rich fraction in maccaroni dough quality. J. Sci. Fd. Agric. 16:233-240 (1965).