COMPARATIVE STUDY OF PENTOSANS IN TRITICALE AND ITS PARENTAL SPECIES

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ABSTRACT

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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