

Physicochemical and Functional Properties of Rye Nonstarch Polysaccharides.

II. Impact of a Fraction Containing Water-Soluble Pentosans and Proteins on Gluten-Starch Loaf Volumes¹

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ABSTRACT

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Rye water-soluble protein-pentosan fractions improve the volume of gluten-starch loaves. A volume-dosage curve shows that the optimum concentration for such substances is 2-3%. The protein component of the rye material appears to have no effect on the capacity of the complex to enhance loaf volume because deproteinization does not result in a loss of the complex's activity. Ferulic acid residues of the water-soluble pentosans of rye or wheat were not involved in the interactions leading

to larger loaf volumes because removal of these moieties or the addition of an excess of ferulic acid in the baking formula did not impair the role of the rye protein-pentosan material in gluten-starch systems. The action of the nonstarch polysaccharides probably is related to an increase in dough viscosity because similar baking results were found when oat β -glucan, corn bran hemicellulose, or xanthan gums were used.

Research on the functional properties of rye hemicelluloses has focused mainly on chemically modified "water-insoluble" pentosans, i.e., substances that are insoluble in their native form but that can be solubilized by treatment with dilute alkaline solutions. Casier and Soenen (1967) and Casier et al (1973) claimed that the addition of 2% of such pentosan material can result in a loaf volume increase of about 30-40%.

These experimental findings were confirmed to a certain extent by Ali and D'Appolonia (1979). These authors prepared pentosans according to the procedure outlined by Casier and Soenen (1967) and Casier et al (1973) and came to the conclusion that bread loaf volume did increase with these materials but to a lesser extent than reported earlier. More recently, Meuser and Suckow (1986) studied the functional properties of both water-soluble and water-insoluble rye pentosans in the baking of rye products. They reported that the water-insoluble pentosans have a negative impact on product characteristics whereas their soluble counterparts have

a beneficial impact on the color, taste, and texture of rye baked goods.

Previous reports (Weipert and Zwingelberg 1979, 1980) have shown that in rye flours there is a definite pentosan-starch ratio of 1:16 at which optimal results in rye breadbaking can be achieved. Although the effect of solubilized rye pentosans in breadmaking with wheat flour and rye flour has been studied, to the best of our knowledge, no reports have been made that deal with the impact of rye water-soluble pentosans in gluten-starch systems, except for a brief mention in the report by D'Appolonia et al (1970).

With wheat flour, Hosenev et al (1969) have established that the removal of the water-soluble pentosans leads to a reduction of loaf volume. Based on their reconstitution experiments, the authors concluded that the addition of excess water-soluble pentosans to reconstituted flours did not result in an increased volume.

Much has been written about the oxidative gelation of wheat pentosans and the involvement of ferulic acid residues in the process. In essence, two different mechanisms have been proposed. Neukom and coworkers (Geissmann and Neukom 1973, Neukom and Markwalder 1978) proposed a mechanism with cross-linkages resulting from oxidative coupling of the aromatic nucleus of ferulic acid moieties esterified to arabinoxylan chains.

Hosenev and Faubion (1981) deduced a second mechanism from their experimental data. According to these authors, the oxidative

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gelation of pentosans involves the addition of a thyl radical to the activated double bond of ferulic acid esterified to the arabinoxylan molecules. A recent report from the same laboratory (Moore et al 1990) has suggested that the mechanism is more complex.

Meuser et al (1982) inferred that at least part of the ferulic acid is esterified directly to protein material and that this material is involved in the oxidative gelation phenomenon. The authors precipitated an arabinoxylan-protein fraction from wheat flour water solubles by adding ethanol to the extract. Further purification of this fraction from both a good- and a poor-bread-making flour by diethylaminoethyl-cellulose chromatography showed that the good-breadmaking flour had a much higher content of the gel-forming fraction. From these data and from gluten-starch loaf baking, the authors concluded that oxidative gelation was involved in breadmaking quality.

Because rye water-soluble protein-pentosan materials also contain ferulic acid residues (Delcour et al 1989b) and undergo oxidative gelation (Nijs 1989), we thought it was important to determine whether that reaction is involved in the mechanism by which these substances lead to an improvement in loaf volume. Therefore, we decided to deesterify the rye arabinoxylan-protein fractions and, thus, remove the ferulic acid. We also wanted to remove the protein component to see whether the ferulic acid and/or the protein material is necessary for the increase in volume ascribed to that fraction.

Thus, as part of a systematic study of the physicochemical and functional properties of rye nonstarch polysaccharides (Delcour et al 1989a), the present effort was undertaken to investigate the effects of water-soluble rye arabinoxylan-protein material on gluten-starch loaf volumes by establishing a volume-dosage relationship, to study whether the protein fractions associated with rye water-soluble pentosans have any influence on their capacity to enhance volume, to gain insight into the role of ferulic acid in the functional properties of pentosan-protein material, and to compare the action of rye arabinoxylan-protein material with that of corresponding fractions from wheat.

MATERIALS AND METHODS

Rye Sample and Rye Milling

The rye sample has been described previously (Delcour et al 1989a). Rye whole meal was used for the extraction of water solubles. First, all broken and nonrye kernels were removed manually from a sample. The clean rye was boiled in ethanol for 60 min to inactivate enzymes. After most of the ethanol had been removed, the sample was dried overnight in an oven at 50°C. The dried sample then was milled with a D.D.D. President laboratory mill (President, Ieper, Belgium).

Extraction of Rye Water-Soluble Pentosan Material

To 3.00 kg of whole-meal rye, 15.0 L of distilled water was added. The rye solids were kept in suspension by mechanical stirring for 240 min at room temperature. The water-soluble fraction was removed from the insoluble material by centrifugation (2,000 × g, 15 min), yielding about 12.0 L of supernatant. To the supernatant, 24.0 L of ethanol (95%) was added, and the mixture was kept overnight in a cold room (4°C). To the water-insoluble rye solids, 7.5 L of water was added, and after 16 hr of mechanical stirring at room temperature, the mixture was centrifuged as above to yield about 7.0 L of supernatant. Ethanol (14.0 L) was added to the supernatant, and it was kept overnight in the cold room (4°C). The precipitated fractions were recovered by centrifugation (2,000 × g, 15 min) and dried by treating the pellets with four aliquots of warm ethanol (50°C) and finally three aliquots of diethyl ether with centrifugation (2,000 × g, 10 min) between each drying step. The products of the two extractions then were air dried, weighed, combined, and well mixed.

Isolation of Wheat Flour Water Solubles and Wheat Water-Soluble Arabinoxylan-Protein Material

Water solubles were separated from wheat flour by shaking a mixture of 1,000 g of flour with 3.0 L of water for 3 min and recovering the water-soluble fraction by centrifugation for 10 min at 1,500 × g. The supernatant then was shell frozen and freeze-dried.

Arabinoxylan-protein material was recovered from wheat flour (500 g) by first shaking the flour with water (1,500 ml) for 3 min. The mixture then was centrifuged for 10 min (1,500 × g), resulting in 1,150 ml of supernatant. To this supernatant, two volumes of ethanol (95%) was added, and the mixture was left overnight in the cold room (4°C). The arabinoxylan-protein material was recovered by centrifugation (10 min, 1,500 × g), and the precipitate was dried by treatment first with alcohol and then with diethyl ether at room temperature, with solvent removal between each drying step.

Isolation of Deesterified or Deproteinized Wheat or Rye Arabinoxylan-Protein Material

Five grams of the above materials was dissolved in 300 ml of 1.0N sodium hydroxide. After 3 (deesterification) or 144 hr (deproteinization) at room temperature (under nitrogen pressure), the solutions were neutralized (pH 7.0) with hydrochloric acid (4.0N) and dialyzed overnight in Spectrapor (Fisher Scientific, St. Louis, MO) membrane tubing (molecular-weight cutoff of 12,000–14,000). The material then was recovered by precipitation with alcohol and subsequent drying with ethanol and ether, as outlined above.

To isolate rye water solubles to be treated with pronase, hand-sorted rye kernels (750 g) were boiled in ethanol for 60 min. The ethanol was removed by drying the sample overnight at room temperature. The sample then was ground with a President laboratory mill. To the whole meal obtained, 3.0 L of water was added. The mixture was kept in suspension for 4 hr in a Hobart mixer (Hobart Brothers Co., Troy, OH). After the first hour of mixing, an additional 750 ml of water was added. The mixture subsequently was centrifuged at 2,375 × g for 15 min. More was removed from the supernatant by centrifuging again at 2,375 × g for 30 min. The supernatant obtained (2.9 L) was divided into two equal parts.

The first part (pH 6.5) was boiled for 10 min, cooled to room temperature, and mixed with two volumes of ethanol (95%). The protein-pentosan material was recovered and dried as described above. This material served as the control for further experiments. To the second 1.45 L of rye extract, pronase (200 units, Sigma P0652, a mixture of nonspecific proteases from *Streptomyces griseus*, Sigma Chemical Co., St. Louis, MO) was added, and the reaction was allowed to proceed for 120 min at 30°C. The mixture then was boiled and treated with ethanol (95%) as outlined above.

Isolation of Corn Bran Hemicellulose

To 100 g of commercially available corn bran (Staley best bran 90 G-fine, A. E. Staley Mfg. Co., Decatur, IL), 1.5 L of 0.5N sodium hydroxide was added. The resulting mixture was stirred with a magnetic stirrer for 24 hr. Then the bran was centrifuged at 2,350 × g for 60 min, resulting in 1,050 ml of supernatant. The supernatant was neutralized to pH 7.0 with a hydrochloric acid solution (4.0N), and the neutralized filtrate was dialyzed overnight in Spectrapor membrane tubing (molecular-weight cutoff of 12,000–14,000). The hemicelluloses were recovered by treating the extract with two volumes of ethanol. The material precipitated overnight (at 4°C) was removed by suction filtration and dried by subsequent aliquots of ethanol and diethyl ether, with solvent removal between each treatment.

Analysis of the Carbohydrate-Protein Fractions from Rye and Wheat

The relative monosaccharide compositions were examined by weighing an aliquot of the sample and submitting it to hydrolysis for the estimation of water-soluble pentosans (Hashimoto et al

1987). The hydrolyzed sample was reduced with sodium borohydride and subsequently acetylated, and the monosaccharide composition was determined by gas chromatography (Delcour et al 1989a). Amino acids were analyzed according to a method described by Khattak and Klopfenstein (1989).

Bread-Baking Materials

Wheat starch and wheat gluten were donated by Midwest Grain Products, Inc., Atchison, KS, and the malted wheat flour was donated by Cargill, Wichita, KS. These materials were analyzed by standard AACC methods (1983). Shortening (Crisco) was purchased from Proctor and Gamble, Cincinnati, OH, and xanthan gum (Keltrol F) was purchased from Kelco, San Diego, CA.

Gluten-Starch Loaf Production

The breadmaking test for 10 g of flour developed by Shogren and Finney (1984) was used throughout this work. All baking trials were performed at least in triplicate. Mixing times were determined at baking and averaged 30–45 sec longer than those determined by the mixograph procedure (Finney and Shogren 1972). Absorption levels were evaluated by the mixograph. Nonfat dried milk (4.0%) and shortening (3.0%) were included in the formula. In the case of gluten-starch loaves, we used 8.10 g of starch and 1.90 g of gluten. Where fractions were to be tested, the starch was diminished such that the total weight of gluten and starch plus the fraction to be studied would still equal 10.0 g (as is).

The fermentation was done with 78.0 mg of Fermipan dried yeast (Gist-brocades, Delft, The Netherlands). Fermentation time

was 180 min, followed by 57 min of proofing. Doughs were punched after 105, 155, and 180 min of fermentation time. We found it useful to place the baked loaves on a wire grid for about 2 hr before determining volumes by dwarf rapeseed displacement.

RESULTS AND DISCUSSION

Isolation of Rye and Wheat Water-Soluble Fractions

The ethanol-precipitated water solubles from rye were obtained as an off-white powder, yield 65.5 g (2.18%). Wheat water solubles were a very light powder, yield 45.0 g (4.50%). An off-white product, 17.7 g (0.89%) from 2,000 g of wheat flour, was isolated from the water by precipitation with ethanol.

The rye water-soluble pentosan fraction had a nitrogen content of 2.14%. The molar ratios of the monosaccharides in this material, taking xylose as 1.00, were 0.71, 0.05, 0.05, and 1.19 (Table I) for arabinose, mannose, galactose, and glucose, respectively. These results agree well with what has been reported earlier (Delcour et al 1989a) and indicate again that the arabinoxylan fraction is highly branched. The recent article by Fengler and Marquardt (1988) showed that 90% of the glucose in similar preparations comes from starch. These analyses showed clearly that an arabinoxylan fraction had been isolated from rye and that it contained protein material. Comparison of the amino acid profiles of the water-soluble protein-pentosan complexes from rye and wheat revealed that these had very similar amino acid compositions (Table II). Furthermore our data on the amino acid contents of wheat water-soluble pentosans agree quite well with the previous data of Meuser et al (1982).

Impact of the Rye Water-Soluble Protein-Pentosan Fraction on Gluten-Starch Loaf Volumes

Volume versus dosage curves. The impact of different levels of rye water-soluble pentosan material (precipitated with ethanol) on the volume of gluten-starch loaves was determined and compared with the effect of a similar fraction prepared from wheat (Fig. 1). The two fractions had similar effects on loaf volume. When the total water-soluble fraction from wheat was used, the loaf volume increased with additions up to about 3% material and remained essentially flat thereafter (Fig. 2). The higher volumes obtained with the total wheat water solubles is explained by the fact that these fractions contain low-molecular-weight nitrogen, which is known to increase loaf volume (Hoseney et al 1969).

D'Appolonia et al (1970) also working with gluten-starch loaves showed that the pentosan fractions from wheat had a volume-enhancing effect. They also reported that the fraction contained protein. Recent work (Meuser et al 1981, Meuser and Suckow 1986) suggested that the fractions used by D'Appolonia et al (1970) contained glycoproteins, i.e., covalently linked pentosan-protein materials. Work by Fincher and Stone (1974) and Yeh et al (1980)

TABLE I
Monosaccharide Composition of the Protein-Pentosan Complexes Isolated from Corn Bran, Rye, and Wheat

Sample	A/X ^a	Gl/X ^a	% (A + X)
RWSP ^b	0.71	1.19	33.0
Deproteinized RWSP (pronase treatment)	0.67	1.13	36.0
Deproteinized RWSP (alkaline treatment)	0.63	0.70	45.4
Deesterified RWSP (moderate alkaline treatment)	0.68	0.77	36.6
WWSP ^c	0.73	0.67	32.0
Deesterified WWSP (moderate alkaline treatment)	0.71	0.50	44.8
Corn bran	0.63	0.04	62.8

^a A, arabinose; X, xylose; Gl, glucose.

^b Rye water-soluble protein-pentosan complex.

^c Wheat water-soluble protein-pentosan complex.

TABLE II
Amino Acid (AA) Content (g/100 g) of the Water-Soluble Protein-Pentosan Fractions from Rye and Wheat

Amino Acid	WWSP ^a	WWSP ^b	RWSP ^c
Aspartic acid	8.4	10.4	8.3
Threonine	4.2	4.6	3.5
Serine	5.0	5.6	6.2
Glutamic acid	22.1	14.8	18.6
Proline	5.2	5.8	7.7
Glycine	6.2	5.9	5.3
Alanine	7.5	6.9	5.9
Half cystine	5.1	1.5	1.9
Valine	5.6	5.0	4.6
Methionine	1.7	1.9	1.6
Isoleucine	2.9	2.9	3.1
Leucine	6.3	8.2	7.1
Tyrosine	1.9	4.5	4.0
Phenylalanine	2.7	5.4	5.5
Histidine	6.6	4.3	4.9
Lysine	2.7	6.1	5.0
Arginine	5.9	6.1	6.7

^a Wheat water-soluble protein-pentosan complex; literature data (Meuser and Suckow 1986).

^b Wheat water-soluble protein-pentosan complex; experimental results.

^c Rye water-soluble protein-pentosan complex.

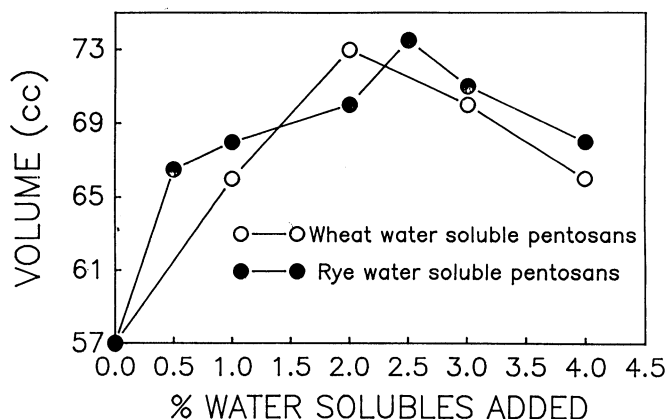


Fig. 1. Volumes (cm³) of gluten-starch loaves (baked according to the procedure outlined in the text) with the addition of different percentages of rye or wheat water-soluble protein-pentosan complex.

clearly showed that the only covalently linked pentosan protein is the fraction soluble in saturated ammonium sulfate. This fraction was identified as an arabinogalactan linked covalently to protein (Fincher and Stone 1974). Meuser et al (1982) and Meuser and Suckow (1986) established that a water extract from wheat, upon treatment with ethanol, results in the precipitation of an arabinoxylan-protein fraction. They also showed that in gluten-starch doughs, this fraction leads to a volume increase.

D'Appolonia et al (1970) included water-soluble materials from rye in their work with gluten-starch loaves. Their results showed that the water-soluble pentosan fractions from rye were able to enhance loaf volume. Those fractions contained protein material; however, their experiments did not show whether the protein moiety was necessary for the fraction's baking performance.

The results obtained with the wheat water solubles suggest that these components do not act independently but through an interaction with the gluten and/or starch. Indeed, such an interaction with other components is the only obvious reason that the volume-enhancement capacity diminishes above certain concentrations.

To gain further insight into the action of the rye material, we decided to bake gluten-starch loaves with the addition of 1% wheat water solubles and 0.5% rye pentosan material, or 5% wheat water solubles and 2.5% rye pentosan material. We hoped to determine whether the rye material would act independently from the effect of the wheat water solubles. For the first series of these loaves, we found an average volume of 71.5 cm³; for the second series, the corresponding figure was 83.0 cm³. Thus, loaf volume increases over the control gluten-starch loaf (having a volume

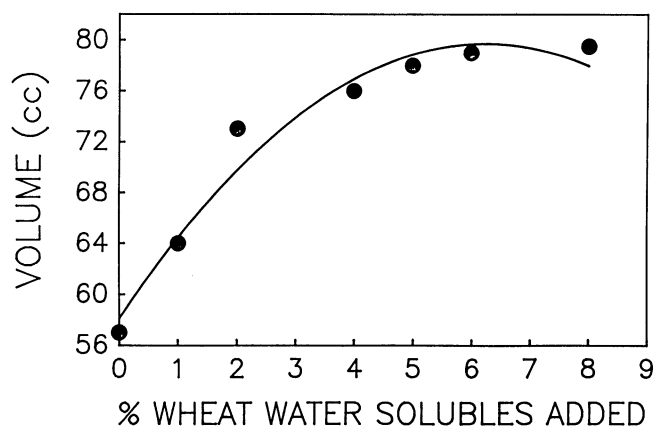


Fig. 2. Volumes (cm³) of gluten-starch loaves (baked according to the procedure outlined in the text) with the addition of different percentages of wheat water solubles.

Table III
Volumes of Gluten-Starch Loaves^a
With or Without 2% of Different Hydrocolloids
and in the Presence or Absence of Ferulic Acid (250 ppm)

Hydrocolloid and/or Ferulic Acid	Volume (cm ³)
None (control)	57.0
RWSP ^b	71.0
WWSP ^c	73.0
Deproteinized RWSP (pronase treatment)	73.0
Deproteinized RWSP (alkaline treatment)	72.5
Deesterified RWSP (alkaline treatment)	69.5
Ferulic acid	64.0
Ferulic acid + RWSP	73.0
Oat β -glucan	62.0
Xanthan gum	64.0
Corn bran hemicellulose	69.5
Deproteinized WWSP (alkaline treatment)	56.0
Deesterified WWSP (moderate alkaline treatment)	68.5

^aBaked according to the procedure outlined in the text.

^bRye water-soluble protein-pentosan complex.

^cWheat water-soluble protein-pentosan complex.

of 57.0 cm³, Table III) were 14.5 and 26 cm³, respectively. If the effects of wheat solubles and rye pentosans had been additive, we would have expected a volume increase of 16.5 cm³ (7.0 + 9.5 cm³, Figs. 1 and 2) and 37.5 cm³, respectively. This indicates that the wheat and the rye material interact in a similar manner because, at lower levels, they have a much more cooperative effect than at higher concentrations.

Effects of Deesterification and Deproteinization

The volume of gluten-starch dough containing no water solubles was 57 cm³ (Table III). Addition of either rye or wheat water-soluble protein-pentosan complex gave a large increase in loaf volume (from 57 to 72 cm³). Deesterification of either the rye or the wheat materials gave only small decreases in the loaf volumes. This would strongly suggest that the gelation of the water-soluble pentosans is not involved in the improvement of loaf volume by the water-soluble fractions. Ferulic acid is known to be involved in the oxidative gelation of the pentosans, and the removal of this entity would stop the gelation. In addition, the presence of free ferulic acid did not decrease loaf volume.

Deproteinization of the water-soluble fraction from rye, either by extended alkaline treatment or by treatment with pronase (Table IV), did not significantly affect loaf volume. Thus, the active component in the rye water solubles appears to be the pentosan. The most likely contribution of pentosans to the dough would be an increase in viscosity. Other gums and fiber sources also would be expected to increase dough viscosity. To show this, we baked gluten and starch doughs containing 2% xanthan gum, oat β -glucan, and 2% of a hemicellulose fraction from corn bran. All of these materials increased the loaf volume compared with gluten-starch alone (Table III).

Deproteinization of the wheat water solubles by an alkaline treatment greatly reduced the loaf volume (Table III). This would indicate that the protein fraction in the wheat water solubles is very important. It is not clear why the fractions from rye and wheat differ in this respect.

CONCLUSIONS

Rye water solubles were shown to contain an arabinoxylan fraction. The fraction also contained protein. The amino acid composition of the rye water solubles was essentially equal to that of a similar fraction from wheat. Addition of water-soluble pentosan material from rye or wheat increased the volume of gluten-starch loaves. Although the effects of such components appeared to be similar, upon deproteinization, the materials reacted differently. More work will be needed to understand these differences.

The present work clearly showed that the well-known oxidative gelation of pentosan material is not involved in the improvement of loaf volume by the pentosan fractions. The fact that the rye water solubles could be deproteinized both by pronase and alkali without altering their baking properties shows that the pentosan material is responsible for the increased volume. These conclusions are supported by the volume-improving effect of oat β -glucan, xanthan, and a fraction from corn bran.

TABLE IV
Amino Acid Content of the Water-Soluble Protein-Pentosan Complex from Wheat and Rye After Different Treatments

Sample	Amino Acid (g/100 g)
RWSP ^a	10.24
Deproteinized RWSP (pronase treatment)	0.45
Deproteinized RWSP (alkaline treatment)	0.94
Deesterified RWSP (moderate alkaline treatment)	8.24
WWSP ^b	28.65
Deesterified WWSP (moderate alkaline treatment)	19.13

^aRye water-soluble protein-pentosan complex.

^bWheat water-soluble protein-pentosan complex.

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