

Effect of Water-Soluble Pentosans on Gluten-Starch Loaves¹

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ABSTRACT

The effect of water-solubles, unfractionated and fractionated pentosans from HRS, durum, and soft wheat endosperm and rye flour on gluten-starch pup loaves was studied. Pentosans were purified and fractionated by alpha-amylase treatment and DEAE-cellulose column chromatography (borate form). Fractions 1 and 2 of the five DEAE-cellulose fractions obtained contained only arabinose and xylose as component sugars with small amounts of protein. Fractions 3, 4, and 5 contained galactose as well as greater amounts of protein. The loaf volume of gluten-starch loaves increased with the addition of water-solubles. The crust color varied, depending on the source of water-solubles. Crude and amylase-treated pentosans extracted from the different sources and added at the 0.8% level to gluten-starch loaves had an improving effect on loaf volume, whereas again crust color varied with the source. Addition of DEAE-cellulose pentosan fractions 1 and 2, essentially pure arabinoxylans, from HRS and soft wheat flour resulted in only a very slight increase, if any, in loaf volume. Addition of the same fractions from durum wheat gave a slightly greater increase, whereas addition of the arabinoxylan fraction from rye flour had no effect. The high protein containing DEAE-cellulose fractions had a definite increasing effect on loaf volume. A detrimental effect on crust color was observed with the addition of the arabinoxylan fractions and was most pronounced with those extracted from soft wheat and rye flour. Grain and texture of gluten-starch loaves with or without additions of water-solubles or pentosans were satisfactory.

The effect of water-soluble material, and water-soluble pentosans in particular, on bread-baking has been studied by different workers.

Sandstedt et al. (1) reported that the water-soluble materials removed in washing were either not essential in baking or exerted their effect before being washed out. In contrast, Finney (2) found that omission of solubles from reconstituted doughs resulted in a definite decrease in loaf volume with two flours but had no effect with a third. Baker et al. (3) found that doughs containing more than the normal level of solubles gave loaves with increased volume. Pence et al. (4) attributed the slight increase in loaf volume with the addition of pentosans to the small amounts of water-soluble protein remaining in the pentosan preparations. In a later paper, Pence et al. (5) showed that a crude albumin fraction was generally responsible for the increased loaf volume. Tracey (6) reported a reduction of loaf volume on addition of snail digestive juice to doughs. This indicated that the pentosans were involved in loaf volume. Cawley (7) found that flour-solubles, which were materials extracted from flour with water followed by heating and dialyzing, when treated with pronase was still effective in improving the volume of gluten-starch loaves. The water-solubles treated with endogenous or snail enzymes were ineffective in improving the volume. Tao and Pomeranz (8) studied the effect of water-soluble pentosan preparations which ranged in protein content from 7.1 to 26.3% in baking. These workers found that pentosans from SRW and especially

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durum flour when added to a flour decreased loaf volume, whereas pentosans from the other flour increased it.

The present study was undertaken in an attempt to help clarify the current role of pentosans in baking. A DEAE-cellulose chromatographic technique was used to fractionate the pentosans, and the effect of the fractions obtained on gluten-starch loaves was examined.

MATERIALS AND METHODS

Samples

The HRS Justin and Thatcher flours and Leeds durum semolina were obtained from a composite of field plot samples grown in North Dakota and milled experimentally on a Buhler mill.

The soft wheat variety, Nugaines, was grown on the west coast and milled on a pilot-scale Miag mill.

The white rye flour was obtained from Bay State Milling Company, Winona, Minn.

A composite of experimentally milled HRS wheats from North Dakota Agricultural Experiment Station field plots served as a source of gluten in the baking.

A commercial sample of unmodified wheat starch, "Starbake" (Hercules, Inc., Harbor Beach, Mich.), was used for the baking.

Isolation of Water-Solubles

Water-soluble material was extracted from the different samples with the use of distilled water. The sample was mixed in a ratio of flour to water (1:2) in a Waring Blendor for 4 min. at low speed. Rye water-solubles required a ratio of flour to water, 1:4. The suspension was centrifuged, the supernatant water-soluble material decanted, shell-frozen, and freeze-dried. Protein content of the various freeze-dried water-solubles was determined by the Kjeldahl procedure (9).

Isolation of Water-Soluble Pentosans

Water-soluble pentosans were extracted from the different samples according to a previous publication from this laboratory, under the sections "Materials and Methods," "Isolation of Water-Soluble Pentosans," and "DEAE-Cellulose Procedure" (10).

Diethylaminoethyl Cellulose Chromatography

Fractionation of the pentosans (1.2 g.) was performed with DEAE-cellulose on a 4 X 50-cm. column as described previously (10). Protein elution during fractionation was followed by a UV monitor and paper strip recorder. Carbohydrate content in the tubes was estimated by the orcinol procedure (11). Protein content in the various freeze-dried fractions recovered from the DEAE-cellulose fractionations was determined by the micro-Kjeldahl procedure (9).

Paper Chromatography

Paper chromatography was performed on the unfractionated and fractionated pentosans as reported in a previous publication (10). Sugars were visualized with silver nitrate spray reagent (12).

Gluten-Starch Baking System

Gluten was hand-washed from a composite of 1966 HRS wheat flours by means

of the dough-kneading procedure (13). A dough was made from 500-g. portions of flour and 300 ml. of 0.1% sodium chloride solution. Starch and soluble material were removed by hand-washing of the dough ball. The freeze-dried gluten was ground on a Wiley mill to pass a No. 30 sieve. The moisture content of the dry gluten was 2.3% and the protein content, dry basis, was 74.2%. The commercial lot of unmodified wheat starch used throughout the baking study contained 8.9% moisture and 0.3% protein.

Physical Dough Properties

The physical dough properties of gluten-starch blends at different protein levels were studied by means of the farinograph and the mixograph (9). A gluten-starch blend of a fixed protein content was selected and used as a control. The effect of adding water-solubles and crude pentosans on the dough properties of such a system was examined.

An indication of the mixing time and mixing tolerance of such a dough system was obtained in this manner.

Baking Study

The effect of water-solubles, unfractionated and fractionated pentosans on gluten-starch loaves was determined with the use of a lean-type baking formula. Gluten-starch blends were baked at different protein levels and one gluten-starch protein level was selected and used throughout the baking study.

Twenty-five-gram pup loaves containing gluten-starch or gluten-starch plus additive were baked. The baking formula was as follows:

Gluten-starch	25 g.
Sugar	8.0% ⁴
Salt	1.0%
Yeast	3.0%
Ammonium phosphate	0.1%
Malt	0.3%
Bromate	0.001%

The temperature of the solutions and samples as well as the temperature and relative humidity of the fermenting doughs was rigidly controlled.

To the gluten-starch sample in the mixing bowl was added: 1 ml. of the ammonium phosphate-bromate solution, 1 ml. of malt solution, 5 ml. of the salt and sugar solution, and 5 ml. of the yeast suspension. Additional water was added to give the dough the proper consistency. The amount of water used was determined by the "feel" of the dough at mixing and also from the feel of the dough of previous bakes at punching and panning. Mixing was performed in a National mixer (National Mfg. Co., Lincoln, Nebr.). A mixing time of 1 min. 20 sec. was used throughout. The mixer was stopped after 30 sec. and the sides and bottom of the mixing bowl were scraped to form a uniform mass of dough. This was necessary since a baking system of this nature did not mix in a manner similar to that for regular flours. The mixing time cited above included the first 30 sec. plus the period after the mixer was restarted.

After mixing the dough was removed from the mixer, rounded into a ball, and

⁴Expressed as % by weight of gluten-starch blend.

placed in the fermentation cabinet. A 3-hr. fermentation and a 55-min. proof period were used. The loaves were baked for 25 min. at 230°C. The volumes were measured by rapeseed displacement 30 min. after removal from the oven. Crust color was judged at the same time with a constant illumination source. The dough characteristics at the panning stage were noted and recorded. The loaves were stored overnight in a cabinet with slight humidification. Crumb grain and texture and crumb color were judged the following day by visual comparison with a standard; a constant illumination source was used.

Water-solubles, unfractionated and fractionated pentosans were added in the dry state. The total water-solubles extracted from the different sources were added at various levels as well as on an equal protein content basis. The effect of unfractionated pentosans as well as the DEAE-cellulose pentosan fractions on gluten-starch loaves was studied at the 0.8% level.

TABLE I. YIELDS AND PROTEIN VALUES OF WATER-SOLUBLES AND PENTOSANS USED IN BAKING

Source	Water-Solubles		Crude Pentosans		Amylase-Treated Pentosans:
	Yield %	Protein %	Yield %	Protein %	Protein %
HRS (Justin)	3.6	26.4	0.79	25.0	23.0
HRS (Thatcher)	3.8	24.8	0.82	24.0	22.2
Durum (Leeds)	4.1	22.2	0.56	22.4	13.8
Soft white (Nugaines)	4.2	17.0	0.76	19.0	21.3
White rye	8.3	25.4	1.85	24.6	23.3

RESULTS AND DISCUSSION

Preparation of Water-Solubles, Unfractionated and Fractionated Pentosans

The yield and protein content of the water-solubles and unfractionated pentosans extracted from different sources and used in the baking study are shown in Table I. The higher yield of white rye water-solubles may be explained in part by the higher flour-to-water ratio used in this extraction. In addition, rye flour is known to contain considerably greater amounts of water-soluble pentosans.

The freeze-dried water-solubles were powdery in appearance, and their color varied from a reddish brown for HRS wheat to a lighter brown for durum and soft white solubles, to a light tan for the rye water-solubles.

Amylase-treated pentosans were recovered in 55 to 65% yield from crude pentosans. The procedure used in treating the pentosans with amylase was such that the amount of protein removed in the trichloroacetic acid step and soluble starch by the amylase enzyme resulted in protein values which were similar for the crude and amylase-treated pentosans.

Five main carbohydrate elution peaks were obtained on fractionation on DEAE-cellulose of the amylase-treated pentosans. Fractions 1 and 2 were essentially arabinoxylans. Fraction 2 was subdivided into two fractions based on the two carbohydrate peaks obtained by orcinol measurement. With the exception

of fraction 4, the remaining fractions were similar in physical appearance to the unfractionated material, being fluffy and cottonlike. Fraction 4 was powdery. This difference in physical appearance was most probably due to the component sugars present in this fraction. This fraction was primarily an arabinogalactan. In all pentosan preparations fractionated on DEAE-cellulose no distinct UV protein absorption peak was observed by the monitor for the first two fractions.

Component sugars in the different fractions as detected by paper chromatography and the ratio as determined by gas-liquid chromatography have been presented in a previous paper from this laboratory (10).

The yield and protein content of the amylase-treated fractions obtained by DEAE-cellulose chromatography are shown in Tables II and III. The data for each

TABLE II. YIELDS AND PROTEIN CONTENT OF DEAE-CELLULOSE FRACTIONS FROM HRS WHEAT FLOUR USED IN BAKING

Fraction	Thatcher				Justin			
	Yield		Protein		Yield		Protein	
	I %	II %	I %	II %	I %	II %	I %	II %
F1	14.3	14.1	2.7	1.8	14.6	15.2	2.1	3.8
F2A	4.3	6.2	4.1	4.3	3.4	4.2	7.8	7.1
F2B	16.3	15.1	2.4	5.1	17.7	16.3	5.5	7.8
F3	21.4	16.6	31.1	35.4	14.9	18.1	45.2	35.8
F4	26.5	29.9	23.8	23.3	36.5	29.5	27.1	22.6
F5	17.3	18.1	17.5	24.7	12.8	16.7	24.0	21.4

sample represent results obtained from two different DEAE-cellulose fractionations of the pentosan in question. This material was used to provide duplicate bakes. Fractions 1 and 2 were the low-protein-containing fractions and, from examination of the paper chromatograms, contained only xylose and arabinose as component sugars. For an unknown reason protein content in fractions 1 and 2 of the soft white and rye pentosans was higher than with the other fractionated pentosans. However, as already mentioned, no main UV protein absorption peak was observed for these fractions. Fraction 1 of the rye pentosans as compared to fraction 1 of the other pentosans was recovered in highest yield. This would be anticipated in view of the higher pentosan content of rye flour. Fraction 1 of the Leeds pentosan as compared to fraction 1 of the other pentosans, on the other hand, was the lowest in yield. In all cases fractions 3, 4, and 5 were high in protein content, and in addition to arabinose and xylose contained galactose as component sugars.

Physical Dough Properties

A gluten-starch blend of 12.3% protein was used throughout the baking study. The mixing time of such a gluten-starch blend as indicated by the mixograph and farinograph was very short, with a high absorption. The low range of stability and short mixing time of the gluten-starch blend can be seen in the farinogram of Fig. 1. The commercial starch preparation used in this study may in part explain the type of farinogram obtained with the gluten-starch blend.

Water-solubles extracted from a flour in a ratio of 2 parts water to 1 part flour

TABLE III. YIELDS AND PROTEIN CONTENT OF DEAE-CELLULOSE FRACTIONS FROM DURUM, SOFT WHITE, AND WHITE RYE USED IN BAKING

Fraction	Durum (Leeds)				Soft White (Nugaines)				White Rye			
	Yield		Protein		Yield		Protein		Yield		Protein	
	I %	II %	I %	II %	I %	II %	I %	II %	I %	II %	I %	II %
F1	9.4	6.1	2.2	4.4	15.4	11.3	5.9	9.4	32.4	34.2	9.5	8.4
F2A	4.1	4.2	4.0	4.0	9.6	12.4	5.1	2.4	11.5	11.3	13.6	13.6
F2B	19.9	21.4	1.8	2.4	11.2	11.2	8.8	9.7	12.9	13.3	...	18.7
F3	11.1	18.7	11.8	14.0	10.8	19.7	42.9	31.8	18.2	19.0	40.7	23.8
F4	36.8	31.5	16.9	16.4	34.4	27.3	25.8	18.2	15.5	13.6	21.5	23.9
F5	19.9	18.0	24.1	23.7	18.5	18.2	22.6	22.2	9.5	8.6	...	13.6

TABLE IV. EFFECT OF WATER-SOLUBLES, PENTOSANS, AND FRACTIONATED PENTOSANS EXTRACTED FROM HRS (THATCHER AND JUSTIN) FLOURS ON GLUTEN-STARCH LOAVES

Addition	Thatcher				Justin			
	Loaf Volume		Crust Color		Loaf Volume		Crust Color	
	I cc.	II cc.	I cc.	II cc.	I cc.	II cc.	I cc.	II cc.
Control (gluten-starch)	127	132	2	2	128	135	2	2
0.8 g. Water-solubles	191	188	3	3	189	170	3	3
0.2 g. Water-solubles	138	155	2+	3	149	148	3	2+
0.2 g. Crude pentosans	142	147	2-	2-	147	154	3	2+
0.2 g. Amylase-treated pentosans	146	145	2-	2-	135	154	2+	2+
0.2 g. F1 + F2A + F2B	135	135	1+	1+	137	136	1+	2+
0.2 g. F3 + F4	147	144	2+	2	135	160	2	3
0.2 g. F4 + F5	153	151	3	2+	158	165	3	2+

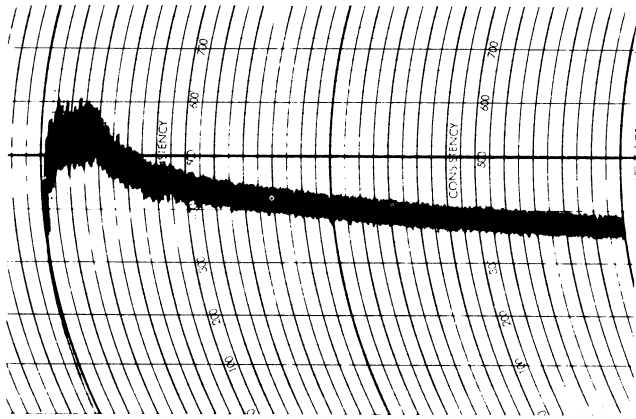


Fig. 1. A farinogram of gluten-starch blend at 12.3% protein.

and added to the gluten-starch system did not affect the mixing time as revealed by the mixograph. The farinogram indicated a slight decrease in absorption with the addition of water-solubles and an increase with the addition of pentosans.

Effect of Water-Solubles, Unfractionated and Fractionated Pentosans on Gluten-Starch Loaves

The baking absorption calculated on a gluten-starch blend of 14% moisture was 74.6%. The gluten-starch doughs mixed with this absorption felt somewhat slack and wet after mixing; however, they recovered during fermentation and felt normal at panning.

In general, as the amount of water-solubles added to the gluten-starch control increased, the greater was the increase in loaf volume. Doughs containing water-solubles at the approximate percentage level recovered from the flour were considerably more extensible than the gluten-starch control.

All gluten-starch loaves showed an increase in loaf volume when water-solubles extracted from the different sources were added at the same percentage basis. The HRS wheat flour water-solubles, however, had the greatest improving effect on loaf volume. Added at the same protein level as the HRS wheat flour water-solubles, Nugaines water-solubles had a similar effect on loaf volume, whereas water-solubles from rye and durum still showed a slightly lower effect on loaf volume.

At the 3.2% level of addition, the water-solubles extracted from different sources had different effects on the crust color of the gluten-starch loaves. Water-solubles from HRS wheat flours improved crust color by giving the crust a darker, more appetizing appearance. Addition of water-solubles from durum wheat also had an improving effect on crust color: not only was the color darker but it was shiny and bright. A detrimental effect on gluten-starch loaves was observed with the presence of water-solubles from either the white rye or Nugaines soft white flour; crust color in these cases was a dull brown.

Crude and amylase-treated pentosans from the different sources added at the 0.8% level to gluten-starch loaves all had an improving effect on loaf volume. The addition of such pentosan preparations as well as the addition of water-solubles

resulted in doughs with better handling characteristics. A slightly higher absorption was used when crude or amylase-treated pentosans were added to the gluten-starch loaves.

Inferior crust color resulted with gluten-starch loaves containing crude pentosans from either Nugaines soft white wheat or white rye. This was particularly evident with the addition of rye pentosans: a very dull brown color resulted. Durum and HRS wheat crude pentosans added at the same level as Nugaines and white rye pentosans did not affect crust color to any extent. As the amount of crude HRS wheat pentosans in the gluten-starch loaves increased, the effect on crust color which was noted at the lower level of added rye pentosans became evident.

The effect of water-solubles, pentosans, and fractionated pentosans from different sources on loaf volume and crust color of gluten-starch loaves is shown in Tables IV and V. Data for each sample are presented for two bakes performed on different days. The fractionated pentosan material used in the two bakes was obtained from two different DEAE-cellulose column fractionations and corresponded to the material in Tables II and III. Crust color was scored on a basis of 1 to 4, number 4 representing very good and number 1, poor.

Table IV shows the results of material extracted from Thatcher and Justin flours on gluten-starch loaves. The addition of 0.8% combined F1 and F2 pentosan fractions from these two hard wheat flour varieties resulted in only a very slight increase in loaf volume. These two fractions, as has been discussed, represent the high-pentosan fractions containing arabinose and xylose as component sugars plus a small amount of protein. The addition of these two fractions also resulted in very dull crust color. Fractions 3 and 4 combined and added at the same level as fractions 1 and 2 showed a greater increase in loaf volume. These two fractions contained considerable protein besides pentosans. The addition of fractions 4 and 5 combined to the gluten-starch loaves had the greatest increasing effect on loaf volume of the combined added fractions. Addition of neither fractions 3 and 4 nor 4 and 5 gave the dull crust color exhibited by the addition of fractions 1 and 2. On the contrary, in most cases, these combined fractions gave an improved crust color.

Table V shows the results of material extracted from durum semolina, soft white, and rye flours on gluten-starch loaves.

An increase in loaf volume was evident with the addition of durum pentosan fractions. In this instance, however, combined fractions 1 and 2, 3 and 4, or 4 and 5 added at the same level showed about the same increase in loaf volume. The effect of the durum pentosan fractions on crust color was evident and showed the same type of pattern as mentioned for the hard wheat flour pentosan fractions.

The same effect on loaf volume with the addition of pentosan fractions from Nugaines soft white flour was obtained as with the addition of HRS wheat flour pentosan fractions. A very slight increase in loaf volume with the high-pentosan combined fractions 1 and 2 and the large increasing effect on loaf volume with combined fractions 4 and 5 was again noted. In addition, pentosan fractions 1 and 2 from Nugaines gave a very pronounced dull crust color. The crust color improved with the addition of either combined fractions 3 and 4 or 4 and 5. The pentosans, it would appear, were responsible for the dull crust color observed with the addition of the crude pentosans.

The addition of pentosan fraction 1 from white rye flour to the starch-gluten control showed a slight decrease in loaf volume. The detrimental effect on crust

color with the addition of crude pentosans or pentosan fractions from rye flour was very evident. Fraction 1 and fractions 1 and 2 combined gave a very, very dull brown color.

Figures 2 and 3 illustrate the effect of water-solubles, unfractionated pentosans,

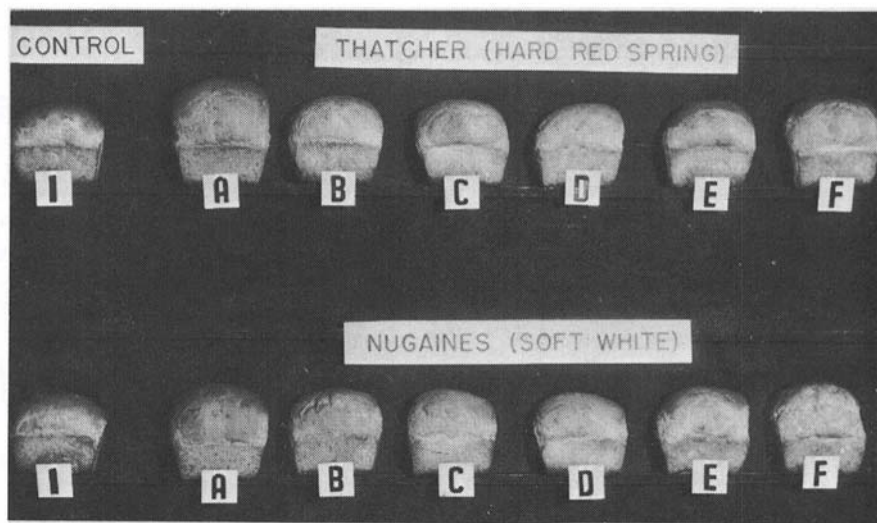


Fig. 2. Effect of water-solubles and pentosans from Thatcher and Nugaines flour on gluten-starch loaves (external view). I, Gluten-starch control; A, 0.8 g. water-solubles; B, 0.2 g. pentosans before alpha-amylase treatment; C, 0.2 g. pentosans after alpha-amylase treatment; D, 0.2 g. F1 + F2A + F2B fractionated pentosans; E, 0.2 g. F3 + F4 fractionated pentosans; F, 0.2 g. F4 + F5 fractionated pentosans.

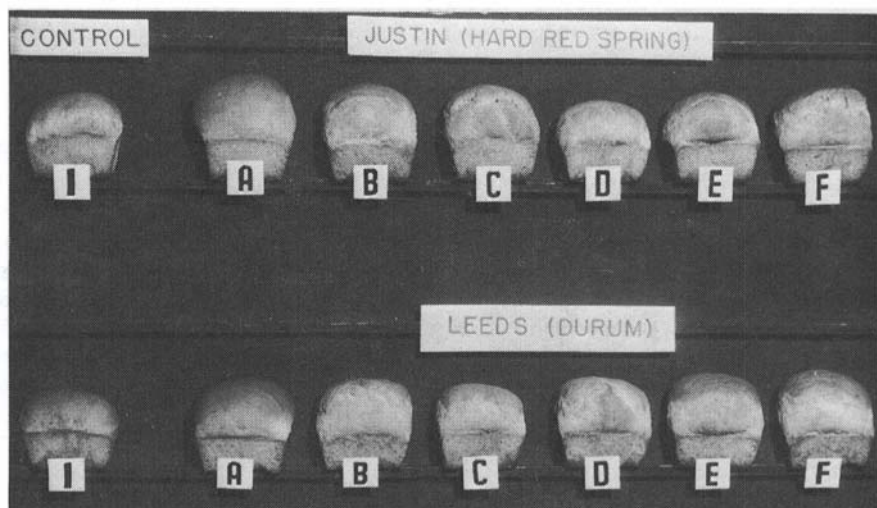


Fig. 3. Effect of water-solubles and pentosans from Justin flour and Leeds semolina on gluten-starch loaves (external view). Legend: same as for Fig. 2.

TABLE V. EFFECT OF WATER-SOLUBLES, PENTOSANS, AND FRACTIONATED PENTOSANS EXTRACTED FROM DURUM (LEEDS) SEMOLINA, SOFT WHITE WHEAT (NUGAINES) FLOUR, AND WHITE RYE FLOUR ON GLUTEN-STARCH LOAVES

Addition	Durum (Leeds)				Soft White (Nugaines)				White Rye			
	Loaf		Crust		Loaf		Crust		Loaf		Crust	
	Volume		Color		Volume		Color		Volume		Color	
	I	II	I	II	I	II	I	II	I	II	I	II
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Control (gluten-starch)	135	136	2	2	128	133	2	2	136	129	2	2
0.8 g. Water-solubles	160	160	3+	3+	160	173	2-	2	172	154	2	1
0.2 g. Water-solubles	142	145	3	3	133	141	2	2+	138	147	2	2
0.2 g. Crude pentosans	155	148	2-	2+	140	141	1+	1+	141	144	1+	1
0.2 g. Amylase-treated pentosans	145	154	2-	2+	135	144	1+	1+	145	135	1+	1
0.2 g. F1 + F2A + F2B	156	150	1+	1+	131	139	1	1				
0.2 g. F3 + F4	164	145	2+	2+	139	155	2+	2+				
0.2 g. F4 + F5	157	159	3	3	158	164	3	3				
0.2 g. F1									131	128	1	1-
0.2 g. F1 + F2A + F2B									138	138	1	1-
0.2 g. F3 + F4 + F5									143	139	1+	1

and pentosan fractions from different sources on gluten-starch loaves. The increasing effect on loaf volume with the addition of combined pentosan fractions 4 and 5 from HRS and soft wheat flours is particularly evident.

In general, the grain and texture of all gluten-starch loaves with or without added water-solubles or pentosans was similar. The main difference was a slightly more open grain with loaves containing material other than gluten and starch. The crumb color of the gluten-starch loaves was also similar in all cases. It was a characteristic trait of such a gluten-starch system to give a slightly whiter crumb than ordinary Buhler-milled flour loaves.

The results of this baking study would tend to support the conclusions of Pence et al. (4), who attributed the slight loaf volume increase in their baking experiments to the small amounts of protein remaining in their pentosan preparations.

With a DEAE-cellulose chromatographic procedure to fractionate pentosans and a gluten-starch baking system, the results would tend to support the idea that the increase in loaf volume of gluten-starch loaves is due to the protein or to a protein-carbohydrate complex and not the pentosans in themselves.

The detrimental effect on crust color of the arabinoxylan fractions was established. The reason for this effect requires further research. One possible explanation is the interference of the pentosans, in some manner, with the browning reaction.

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