Effects of (1→3)(1→4)-β-D-Glucans of Wheat Flour on Breadmaking[1]

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ABSTRACT

Water-soluble nonstarch polysaccharides were extracted from commercial hard red winter wheat flour and separated into three fractions by graded ethanol precipitation. The three fractions, F15, F40, and F60, varied in polysaccharide composition. Fraction F15 was rich in water-soluble (1→3)(1→4)-β-D-glucans, and fractions F40 and F60 were rich in arabinoxylans. Addition of individual fractions to a bread formula did not affect bread loaf volume. Addition of fraction F15 to the formula improved bread crumb grain. Treatment of (1→3)(1→4)-β-D-glucan–rich fraction F15 with lichenase before its addition to the bread formula resulted in bread with poor crumb grain. Treatment of the F15 fraction with β-xylanase before its addition to the bread formula resulted in bread with slightly improved crumb grain. Presumably, the (1→3)(1→4)-β-D-glucans in fraction F15 improved crumb grain by stabilizing air cells in the bread dough and preventing coalescence of the cells. Addition of pentosan-rich fractions F40 and F60 to the bread formula did not improve crumb grain and interfered with the improving effect of (1→3)(1→4)-β-D-glucan–rich fraction F15. Hydrolysis of the arabinoxylans in flour by adding β-xylanase to the bread formula resulted in improved crumb grain.

Wheat endosperm cell walls consist predominantly of arabinoxylans and (1→3)(1→4)-β-D-glucans. Some of these polysaccharides are water-soluble. Water-soluble polysaccharides play an important role in breadmaking. Hoseney et al (1969) reported that nondialyzable water-soluble fractions containing pentosans and glycoproteins appear to contribute to gas retention and gluten extensibility. The role of pentosans in breadmaking has been studied; however, few studies of (1→3)(1→4)-β-D-glucans from wheat have been reported.

Water-soluble (1→3)(1→4)-β-D-glucans may contribute to water absorption of doughs because their asymmetrical "rod- or worm-like" configuration in aqueous solution allows them to absorb large amounts of water (Fincher and Stone 1986). Several studies have suggested that substances with high water-binding capacity and high solution viscosity are important influences on flour water absorption, dough mixing, dough rheological properties, and bread characteristics (Jelaca and Hlynka 1972, Holas and Tipples 1978, Shelton and D'Appolonia 1985, McCleary 1986, Michniewicz et al 1990). Drews (1979) reported that highly hydratable substances, mainly β-glucans, affect rye flour baking quality and that addition of β-glucanase has adverse affects on rye bread crumb quality. Delcour et al (1991) reported that addition of oat β-glucan increases the volume of gluten-starch loaves, most likely by increasing the viscosity of the dough. No work has been reported concerning the effect of endogenous wheat (1→3)(1→4)-β-D-glucans on breadmaking.

Crumb grain is an important bread characteristic. Generally, bread with good loaf volume will have satisfactory crumb grain. However, in recent years, crumb grain quality of commercial bread has declined, while volume has gradually improved, suggesting bread crumb grain is controlled independently of loaf volume by undocumented factors. The purpose of this study was to determine whether there is a relationship between (1→3)(1→4)-β-D-glucans and bread characteristics, particularly crumb grain.

MATERIALS AND METHODS

Flour

A hard red winter commercial bread flour was used for extraction and fractionation of polysaccharides and bread-making tests. The flour contained 13.0% moisture and 10.5% protein (flour weight basis [fwb], as is). Before extraction of nonstarch polysaccharides, the flour was heated for 90 min at 130°C to inactivate endogenous enzymes.

Estimation of Endogenous Enzyme Activity of the Flour

The activity of endogenous α-amylase enzymes in flour, before and after heat treatment, were measured with a Rapid ViscoAnalyzer (RVA). The RVA temperature profile was linear. The following temperatures were reached after the specified times: 65°C (2 min), 95°C (4 min), held at 95°C (6 min), 50°C (13 min), and held at 50°C (6 min).

The activities of endogenous (1→3)(1→4)-β-D-glucan–hydrolyzing enzymes (β-glucanase, cellulase, etc.) were estimated by an enzymatic method with a β-glucanase assay kit provided by Megazyme International Ireland, Ltd. (Wicklow, Ireland). The estimation followed the procedure described in the brochure supplied with the Megazyme malt β-glucanase assay procedure kit. The procedure was modified to use a 2.5-g wheat flour sample instead of the 0.5-g sample called for in the method. A standard curve provided by Megazyme ($Y = 650 \times \text{absorbance} + 45$) was used for enzyme activity calculation. Results were corrected with the β-glucanase activity value of the barley standard.

The activity of (1→3)(1→4)-β-D-glucan–hydrolyzing enzymes in a β-xylanase sample (Megazyme) also was estimated by the method described above. Xylanase (400 and 800 units) was mixed directly with a 0.5-mL β-glucan standard (Megazyme).

Extraction and Fractionation of Nonstarch Polysaccharides

Water-soluble polysaccharides were extracted and fractionated following the ethanol precipitation method described by Cleemput et al (1994), with some modifications (Fig. 1). Flour was heated for 90 min at 130°C to inactivate endogenous flour enzymes. Water-solubles were extracted with water at a flour-to-water ratio of 1:5 at room temperature and heated to 90°C to denature soluble proteins, which were removed by centrifugation. Soluble starch in flour water-soluble polysaccharides was hydrolyzed at 90°C with a heat-stable bacterial α-amylase (EC 3.2.1.1, from *Bacillus licheniformis* [Sigma Chemical Company, St. Louis, MO]). This enzyme and temperature combination was used to eliminate any β-glucan–hydrolyzing enzyme activity.

The solution was made to 65% ethanol (v/v), and the mixture was stirred for 30 min and held overnight at 4°C. The mixture was centrifuged at 6,000 × *g* for 30 min at 4°C. The precipitate was redissolved in water, and alcohol was precipitated with 60% ethanol. The precipitate obtained was dried by washing with ethanol (2×) and acetone. The result was the water-soluble nonstarch polysaccharide (WNSP) fraction.

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The WNSP fraction was subfractionated, as shown in Fig. 2; it was dissolved in water, and ethanol was added to make the solution 15% ethanol. The mixture was allowed to set overnight at 4°C and centrifuged at 6,000 × *g* for 30 min. The precipitate was resuspended in water and lyophilized to produce the F15 fraction. The supernatant was made to 40% ethanol, allowed to set overnight at 4°C, and centrifuged at 6,000 × *g* for 30 min. The precipitate was resuspended in water and lyophilized to produce the F40 fraction. The resulting supernatant was made to 60% ethanol, allowed to set overnight at 4°C, and centrifuged at 6,000 × *g* for 30 min. The precipitate was resuspended in water and lyophilized to produce the F60 fraction.

Enzyme Treatment of Fractions

Lichenase (*endo*-1,3(4)-β-D-glucanase, EC 3.2.1.73, from *B. subtilis* [Megazyme]) and β-xylanase (EC 3.2.1.8, from *Trichoderma viride* [Megazyme]) were used separately to remove β-glucans and arabinoxylans, respectively, from fractions F15 and F40.

For lichenase treatment, the F15 fraction (0.50 g) was dissolved in 1,000 mL of sodium phosphate buffer (20m*M*, pH 6.5) and treated with 80 units of lichenase at 40°C for 1 hr. The temperature was raised to 95°C for 25 min to stop enzyme action. After the mixture was cooled to room temperature, ethanol was added until the mixture was 65% ethanol (v/v), and the mixture was stirred for 30 min. The mixture was stored overnight at 4°C and centrifuged at 10,000 × *g* for 30 min, after which the supernatant was discarded. The insoluble fraction was dissolved in water, dialyzed against distilled water for 48 hr, and lyophilized to produce F15L (fraction F15 treated with lichenase).

Both F15 (0.50 g/1,000 mL of buffer) and F40 (0.50 g/500 mL of buffer) fractions were dissolved in sodium acetate buffer (30m*M*, pH 5.0) and treated with 80 units of β-xylanase. Treatment conditions and recovery of fractions were as described above for the lichenase treatment. F15X and F40X samples were produced by β-xylanase treatment.

Analysis of Saccharides

Total carbohydrate content was estimated by the phenol-sulfuric acid procedure (Dubois et al 1956), with absorption at 490 nm and glucose as a standard. For HPLC analysis, polysaccharide fractions were hydrolyzed with 1*N* sulfuric acid at 100°C for 3 hr. After hydrolysis, barium hydroxide was added to pH 7.0 to neutralize the solution. The samples were filtered through glass-fiber filter paper (grade 934AH, Whatman) to remove the barium sulfate precipitate.

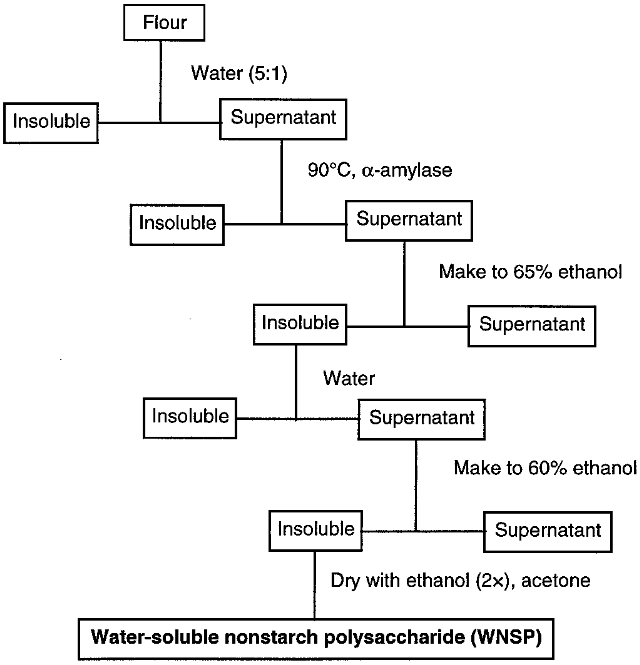

Fig. 1. Extraction and fractionation of water-soluble nonstarch polysaccharides.

The monosaccharide composition and concentration of hydrolyzates was determined with a Dionex (Marlton, NJ) system and pulsed amperometric detector (HPLC-PAD). A CarboPac PA1 analytical column (4 × 250 mm) with a CarboPac PA Guard column (3 × 25 mm) was used. The column was maintained at 25°C and developed with a mixture of 24m*M* sodium hydroxide and 1.6m*M* sodium acetate at a flow rate of 1.0 mL/min. Pulse potentials (volts) and duration (seconds) on the PAD were $E_1 = 0.05$ ($t_1 = 0$); $E_2 = 0.05$ ($t_2 = 0.50$); $E_3 = 0.6$ ($t_3 = 0.51$); $E_4 = 0.6$ ($t_4 = 0.59$); $E_5 = -0.60$ ($t_5 = 0.60$); and $E_6 = -0.06$ ($t_6 = 0.65$). Samples were passed through IR-120, filtered with Supor Acrodisc syringe filters (0.45-µm pores) (Gelman Sciences, Ann Arbor, MI), and injected through a 20-µL injection loop. Chromatograms were recorded on a Chromatopac LR 601 digital integrator (Shimadzu Scientific Instruments, Columbia, MD). The average peak area of two injections was used to calculate the concentration of monosaccharides.

A standard curve was prepared with a series of solutions of D-arabinose, D-galactose, D-glucose, D-xylose, and D-ribose. The concentrations of the standards were 0.54, 0.90, 1.8, 3.6, and 5.4 µg/mL for glucose and galactose and 0.45, 0.75, 1.50, 3.00, and 4.50 µg/mL for arabinose and xylose. D-ribose was used as an internal standard (3.00 µg/mL) for standards and all test samples.

Determination of Protein Content

Protein content (N × 5.7) of fractions was determined by Approved Method 46-13, the micro-Kjeldahl method (AACC 1995) using methyl red-methylene blue indicator. The bicinchoninic acid procedure (Smith et al 1985) also was used to measure the protein content of certain samples.

Breadmaking

Pup loaf bread was produced by Approved Method 10-10B, the 90-min fermentation straight-dough method (AACC 1995). Water absorption, $KBrO_3$ level, and mixing time were optimized for the control flour and kept constant for all treatments. All breads were produced in triplicate. Loaf volume of bread was measured by rapeseed displacement. After cooling, bread was sliced and subjectively scored for internal quality characteristics. Bread with satisfactory crumb grain had medium, elongated cells with thin cell walls. Bread with superior crumb grain had small, elongated cells with thin cell walls. Bread with poor crumb grain had many large, round cells with thick cell walls or many small, round cells with thin cell walls.

For baking trials, three separate enzyme preparations were added to the pup loaf bread formula: lichenase (*endo*-1,3(4)-β-D-glucanase, EC 3.2.1.73, from *B. Subtilis* [Megazyme]), commercial

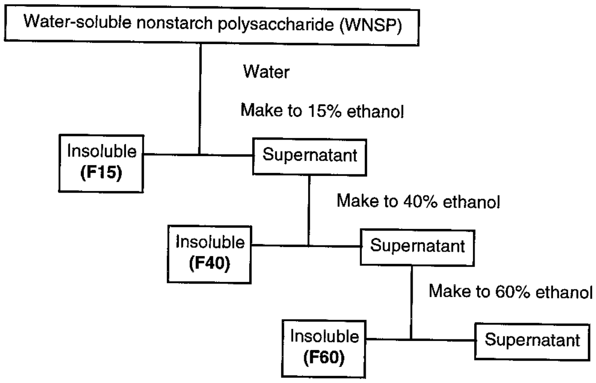

Fig. 2. Subfractionation of water-soluble nonstarch polysaccharides to produce fractions F15, F40, and F60.

hemicellulase (Veron He, Rohm Tech, Somerset, NJ) containing β-(1,4)-glucan endohydrolase activity (EC 3.2.1.4), and an endo-cellulase (*endo*-1,4-β-D-glucanase, EC 3.2.1.4., from *T. viride* [Megazyme]). Endo-cellulase and lichenase were dissolved in dough water: lichenase at 5, 6, and 7 units/100 g of flour and endo-cellulase at 2.0, 2.5, and 3.0 units/100 g of flour. Veron He was dry blended with flour (0.01 and 0.02 g/100 g of flour). The commercial preparation did not specify the units of activity. All treatments were baked in triplicate.

Objective Evaluation of Bread Crumb Grain

A computerized image analyzing system (Shen 1996) was used for objective evaluation of bread crumb grain. Two slices of fresh-cut bread from each loaf were analyzed. Images of bread crumb were obtained with a Panasonic TV camera. Care was taken to obtain the image from the same position on each slice of bread. Size, perimeter, and roundness of bread cells in an area of 450 × 450 pixels on the monitor were measured. An image of a sheet of engineering graph paper was obtained and used to convert pixels into square millimeters.

RESULTS AND DISCUSSION

Endogenous Enzyme Activity of Flour

The flour had an average RVA stirring number of 36.5. Heat treatment raised the RVA stirring number to 303, indicating the amylase had been inactivated.

The activity of β-glucan–hydrolyzing enzymes (mainly β-glucanase and cellulase) was low in the commercial flour. When a 0.5-g flour sample was used for testing, as recommended by the standard test procedure, enzyme activity was undetectable. Low levels of enzyme activity were detected when the sample size was increased to 2.5 g (19.8 ± 0.85 units/kg). No β-glucan–hydrolyzing enzyme activity was detected in heat-treated flour, even when flour weight was increased to 2.5 g.

Composition of Different Fractions

Fraction WNSP. After removing denaturable protein and soluble starch, WNSP was precipitated with 65% ethanol. Precipitated, dried WNSP accounted for ≈0.6–0.7% of the flour (dwb) and predominately contained arabinoxylans (Table I). Extracted substances containing glucopyranose units, presumably from β-glucans, accounted for ≈6% of the WNSP or ≈0.04% of the dry flour weight. Only small amounts of mannose and galactose were found. The low level of galactose indicates arabinogalactans were not part of the preparation. These results agree well with previously reported water-extractable polysaccharide composition (Cleemput et al 1993, 1994).

Fraction F15. The WNSP fraction was fractionated further by dissolving it in water and precipitating with different concentrations of ethanol. Fraction F15 accounted for ≈0.04–0.05% of the dry flour weight. Approximately 46.8% of the dry weight of this fraction was glucose (Table I). A small amount of arabinoxylans and ≈15% protein also were present. The glucans and arabinoxylans in this fraction probably were higher in molecular weight because they were precipitated with a low concentration of ethanol. Woodward et al (1983) reported that β-glucans with higher molecular weights could be precipitated easily with ethanol.

TABLE I
Yield and Monosaccharide Composition of Fractions (F15, F40, and F60) Precipitated by Ethanol

Composition	WNSP[a]	F15	F40	F60
Yield (%)[b]	0.64 ± 0.02	0.046 ± 0.003	0.35 ± 0.04	0.20 ± 0.01
Monosaccharide[c]				
Arabinose	30.40 ± 0.78	7.51 ± 0.79	25.96 ± 0.21	40.06 ± 0.56
Xylose	54.43 ± 0.77	17.84 ± 0.46	51.74 ± 0.56	52.80 ± 0.36
Mannose	0.08 ± 0.01	Trace	Trace	0.12 ± 0.04
Galactose	1.12 ± 0.12	1.18 ± 0.18	0.23 ± 0.07	0.75 ± 0.15
Glucose	5.62 ± 0.58	46.80 ± 0.5	4.17 ± 0.53	2.70 ± 0.30

[a] Water-soluble nonstarch polysaccharides.
[b] Percent (w/w) based on flour dry weight. Mean ± standard deviation ($n = 3$).
[c] Percent (w/w) based on fraction dry weight. Mean ± standard deviation ($n = 3$).

When the F15 fraction was treated with lichenase (from *B. subtilis*) to remove the β-glucans and dialyzed against distilled water, the remaining polysaccharides (fraction F15L) contained only a trace of glucose (Table II), indicating the original fraction contained mostly β-glucans. The small amount of glucose that remained after lichenase treatment may be from resistant starch (Delcour and Eerlingen 1996), nonmixed linkage β-glucans, or incompletely hydrolyzed β-glucans. β-Xylanase, which hydrolyzes arabinoxylans, also was used to treat the F15 fraction. The fraction after treatment (F15X) contained a high percentage of glucans but essentially no arabinoxylans (Table II).

To determine the monosaccharide composition, the polymers were subjected to acid hydrolysis. The recovery rate for the fractions after acid hydrolysis was ≈90% by weight, as estimated by the phenol-sulfuric method (Table III). Hydrolysis loss is an inherent problem in monosaccharide analyses (Dutton 1973). Regardless of the method used, substantial and differential losses of monosaccharides may occur during acid hydrolysis (Fincher and Stone 1986).

Fraction F40. Fraction F40 predominately contained arabinoxylans, with a small percentage of glucans (Table I). The yield of this fraction was ≈0.3–0.4% of the flour dry weight. The glucans and arabinoxylans in this fraction presumably have a lower molecular weight than those in the F15 fraction, because a higher concentration of ethanol was required to precipitate them. Treatment with β-xylanase removed most of the arabinoxylans to yield a fraction (F40X) containing a high percentage of glucans (Table II).

Fraction F60. Fraction F60 accounted for ≈0.2% of the dry flour weight. Most of the polysaccharides in this fraction were

TABLE II
Yield and Monosaccharide Composition of Fractions Obtained by Enzyme Treatments

Composition	F15X[a]	F15L[b]	F40X[c]
Yield (%)[d]	42.0 ± 0.90	46.0 ± 0.80	8.5 ± 0.30
Monosaccharide[e]			
Arabinose	2.97 ± 0.09	12.62 ± 0.78	2.84 ± 0.29
Xylose	0.00 ± 0.03	29.18 ± 0.22	1.46 ± 0.26
Mannose	Trace	Trace	Trace
Galactose	Trace	Trace	Trace
Glucose	48.40 ± 1.2	5.50 ± 0.27	33.03 ± 0.43
Protein[e]	18.40 ± 0.18	23.01 ± 0.20	30.60 ± 0.16

[a] Fraction F15 treated with β-xylanase.
[b] Fraction F15 treated with lichenase.
[c] Fraction F40 treated with β-xylanase.
[d] Percent (w/w) based on dry weight of F15 or F40. Mean ± standard deviation ($n = 2$).
[e] Percent (w/w) based on dry weight of F15L, F15X, or F40X. Mean ± standard deviation ($n = 2$).

TABLE III
Carbohydrate Content[a] and Hydrolysis Recovery of Fractions

Content and Recovery	F15[b]	F15X[c]	F15L[d]
Carbohydrate (%)[e]			
Before hydrolysis	86.9 ± 0.4	77.2 ± 0.2	76.0 ± 0.3
After hydrolysis	77.9 ± 0.3	68.7 ± 0.4	71.4 ± 0.2
Hydrolysis recovery (%)[f]	89.6 ± 0.3	89.0 ± 0.2	94.0 ± 0.2

[a] Measured by phenol-sulfuric acid method with β-D-glucose as standard at 490 nm.
[b] Untreated fraction F15.
[c] Fraction F15 treated with β-xylanase.
[d] Fraction F15 treated with lichenase.
[e] Percent (w/w) based on dry fraction weight. Mean ± standard deviation ($n = 2$).
[f] Percent (w/w) of total carbohydrates after acid-hydrolysis over total carbohydrates before acid-hydrolysis. Mean ± standard deviation ($n = 2$).

arabinoxylans. Glucans and other substances containing glucopyranose units were found in small amounts (Table I).

Effect of Enzyme Treatments on Baking

Addition of lichenase, endo-cellulase, or hemicellulase (Veron HE) to the bread formula caused significant deterioration of the crumb grain of the resultant bread (Table IV) but did not change loaf volume. At higher levels, enzymes were not as detrimental. The reason for this was unclear.

Addition of β-xylanase at 5 or 10 units/100 g of flour did not affect loaf volume but slightly improved bread crumb grain (data not shown). This agrees with the results of Baez-Vasquez and Schofield (1993), Rouau et al (1994), and Qi Si (1996), who all reported that when xylanase was used at a low dosage, it provided good dough extensibility, larger bread volume, and improved crumb structure.

Effect of Different Polysaccharide Fractions on Baking

Effect on bread loaf volume. Loaves containing WNSP, F15, F40, and F60 fractions or a reconstitution of the subfractions (F15 + F40 + F60) did not show significant differences in volume compared to loaves made with the control flour (Table V).

Effect on bread crumb grain. Addition of fraction F15 (0.09% fwb) significantly improved bread crumb grain over that of unsupplemented bread. (Table V). The crumb grain was fine, with elongated cells and thin cell walls. Crumb grain image analysis showed that bread containing F15 had cells with significantly lower mean size and roundness (Table VI). Addition of fraction F40 at a lower level (0.3% fwb) provided marginal improvement in crumb grain but was not as effective as the F15 fraction. The F60 fraction also caused a minor improvement in crumb grain (Table V). WNSP and the combination of fractions F15, F40, and F60 produced bread with essentially the same crumb grain as the control. The beneficial effect of fraction F15 appeared to be overcome by the presence of the F40 and F60 fractions.

Fractions WNSP; F15 + F40 + F60; F15 + F40; F40; and F60 predominantly contained pentosans and did not have significant effects on bread crumb grain. The effects of fractions F15L, F15X, and F40X are shown in Table VII. Fraction F40X showed no improvement in bread crumb grain at either treatment level. Addition of arabinoxylan-rich fraction F15L did not improve bread crumb grain at 0.025% and resulted in poorer crumb grain at 0.05%. Fraction F15X evaluated subjectively slightly improved bread crumb grain when added at 0.06% fwb. Data from crumb grain image analysis indicated that bread supplemented with fraction F15X had significantly better crumb grain, with lower mean cell size and less roundness (finer, more elongated cells) than the control and all fractions, except fraction F15 (Table VI).

Effect of (1→3)(1→4)-β-D-Glucans

Fraction F15 always significantly improved crumb grain, producing a crumb grain with finer and more elongated cells. Fraction F15 was rich in (1→3)(1→4)-β-D-glucans but also contained significant amounts of arabinoxylans. Fraction F15X, which was rich in (1→3)(1→4)-β-D-glucans but did not contain arabinoxylans, also improved crumb grain (Table VI). When the (1→3)(1→4)-β-D-glucans in F15 were removed, the resulting fraction (F15L) produced bread with slightly poorer crumb grain. Thus, the (1→3)(1→4)-β-D-glucans seemed to be critical for the crumb grain improvement effect of F15.

The (1→3)(1→4)-β-D-glucans in fractions F40 and F40X did not improve crumb grain. Thus, it appeared that the (1→3)(1→4)-β-D-glucans with higher molecular weights (F15) were more effective at improving crumb grain. β-Glucans in the F15X fraction may be significantly lower in molecular weights because they may be degraded by enzymes present in the xylanase. This may partially explain why the improvement effect of fraction F15X was not as significant at the 0.03% level as at 0.06%.

TABLE IV
Effect of Enzymes on Bread Loaf Volume and Crumb Grain

Enzyme	Treatment Level (units)	Loaf Volume[a] (cm^3)	Crumb Grain[b]
Control	0.0	920c	S
Lichenase	5.0	932a–c	P
	6.0	925a–c	P
	7.0	926a–c	S–
Endo-cellulase	2.0	940ab	P
	2.5	957a	P
	3.0	902c	S–
Veron He[c]	0.01	898c	S–
	0.02	943ab	S–

[a] Means followed by the same letter are not significantly different ($P < 0.05$).
[b] S = satisfactory: medium, elongated cells with thin cell walls; P = poor: many large, round cells with thick cell walls or many small, round cells with thin cell walls; S– = slightly less than satisfactory.
[c] Veron He: hemicellulase, units/gram unknown.

TABLE V
Effect of Fractions on Bread Loaf Volume

Fraction	Fraction Supplement Level (% flour weight)	Mean of Loaf Volume[a] (cm^3)	Crumb Grain[b]
Control	0.00	863	S
WNSP[c]	0.60	862	S+
F15	0.09	828	Sp
F40	0.30	852	S+
F60	0.19	830	S+
F15 + F40 + F60	0.09 + 0.30 + 0.19	860	S

[a] Means ($n = 3$) are not significantly different ($P > 0.05$).
[b] S = satisfactory: medium, elongated cells with thin cell walls; Sp = superior: small, elongated cells with thin cell walls; S+ = slightly better than satisfactory.
[c] Water-soluble nonstarch polysaccharides.

TABLE VI
Objective Evaluation of Crumb Grain of Supplemented Bread

Fraction	Fraction Supplement (% flour weight)	Cell Size[a] (pixels)	Cell Roundness[b] (width/length)
Control	0.000	41.08a	0.778a
F15	0.040	37.87d–f	0.720c–f
	0.080	37.35ef	0.693ef
F15X[c]	0.020	37.38ef	0.677fg
	0.040	36.29f	0.643g
F15L[d]	0.025	39.66a–c	0.760a–c
	0.050	40.02a–c	0.778a

[a] Mean ($n = 2$). LSD = 1.76. Means followed by the same letter are not significantly different ($P > 0.05$). Pixel = 19.7 × 10^{-3} mm^2.
[b] Mean ($n = 2$). LSD = 0.045. Means followed by same letter are not significantly different ($P > 0.05$).
[c] Fraction F15 treated with β-xylanase.
[d] Fraction F15 treated with lichenase.

TABLE VII
Effect of Fractions on Internal Characteristics of Bread

Fraction	Fraction Supplement Level (% flour weight)	Crumb Grain[a]
Control	0.00	S
F15X[b]	0.03	S
	0.06	S+
F15L[c]	0.025	S
	0.05	S–
F40X[d]	0.03	S
	0.06	S

[a] S = satisfactory: medium, elongated cells with thin cell walls; S+ = slightly better than satisfactory; S– = slightly less than satisfactory.
[b] Fraction F15 treated with β-xylanase.
[c] Fraction F15 treated with lichenase.
[d] Fraction F40 treated with β-xylanase.

The (1→3)(1→4)-β-D-glucans in fraction F15 appeared to improve crumb grain by stabilizing air cells in the bread dough and preventing coalescence of the cells. As a result, the mean cell size in the bread containing fraction F15 was smaller (Table VI). The (1→3)(1→4)-β-D-glucans have a high molecular weight and may exist as long-chain worm- or rod-like molecules in the aqueous phase of dough, as in other aqueous solutions. They are also a fibrous material with a large surface area per unit of weight, which may allow them to improve the extensibility of dough and stabilize gas cells, perhaps by structuring a large amount of water in the dough phase and improving the mechanical strength of the liquid film (Gan et al 1995) or by associating with the continuous protein phase in the dough (Bushuk and MacRitchie 1988). As a result, the dough phase joining the gas cells is able to stretch further without rupturing, providing bread crumb with thinner cell walls.

Effect of Other Compounds

No evidence indicates that compounds other than the arabinoxylans and β-glucans are functional in improving crumb grain. Fraction F15 contained denatured protein that also appeared in fractions F15L and F15X. However, these fractions functioned differently in improving crumb grain.

CONCLUSIONS

Addition of nonstarch polysaccharide fraction F15 from the water-soluble polysaccharides of wheat flour significantly improved crumb grain of bread. Fraction F15 was rich in (1→3)(1→4)-β-D-glucans but also contained arabinoxylans and denatured proteins. The (1→3)(1→4)-β-D-glucans appeared to be critical for the crumb grain improvement effect of fraction F15. Removing the (1→3) (1→4)-β-D-glucans with enzymes (F15L) resulted in loss of this improvement, whereas removal of arabinoxylans from fraction F15 did not result in loss of improvement. Presumably, the (1→3) (1→4)-β-D-glucans in fraction F15 improved crumb grain by stabilizing air cells in the bread dough and preventing coalescence of the cells.

Water-soluble pentosans did not have a beneficial effect on crumb grain. Addition of pentosan-rich fractions F40 and F60 did not improve crumb grain and interfered with the improving effect of (1→3) (1→4)-β-D-glucan–rich fraction F15. Addition of β-xylanase to the bread formula to hydrolyze the arabinoxylans improved crumb grain.

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