Extraction and Enrichment of (1→3),(1→4)-β-D-Glucan from Barley and Oat Brans

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ABSTRACT

β-Glucan was extracted and purified from Tupper barley bran (6.6% β-glucan), Azhul barley bran (13.4% β-glucan), and commercial oat bran (6.9% β-glucan) with solvent 1 and, in the case of Azhul barley bran, with two additional solvents (2 and 3). Solvent 1 was distilled water adjusted to pH 10 with 20% sodium carbonate, solvent 2 was distilled water adjusted to pH 7, and solvent 3 was 4% (w/v) sodium hydroxide. Solvent 1 extracted 61–64% of the total β-glucan from the barley brans and 70% from oat bran; solvents 2 and 3 extracted 72 and 84%, respectively, from Azhul barley bran. The final yields, in percent β-glucan recovered from barley brans, were solvent 1, 52–55%; solvent 2, 40%; solvent 3, 81%. The yield from oat bran with solvent 1 was 61%. The purified preparations contained 72–81% β-glucan + pentosans from barley brans and 84% from oat bran, expressed on an ash-free basis. They also contained 0.2–0.5% total nitrogen, 0.4–1.2% starch, 1.1–10.9% pentosans, and 3.7–12.6% ash. Ether extract was not detected in any of the preparations. Enrichment index, calculated by percent yield × (percent β-glucan + pentosan, ash-free basis)/100, was highest for solvent 3 (65), lowest for solvent 2 (33), and varied from 38 to 51 for solvent 1. Sodium hydroxide (solvent 3) appeared to be a better solvent for extraction and purification of β-glucan from barley bran. Size-exclusion chromatography of the three β-glucan preparations from Azhul barley bran suggested an apparent molecular weight of about 2×10^6. Molecular weights of the preparations were not correlated with their flow viscosity.

Barley, particularly genotypes having waxy (low amylose) starch, contains, unlike oats, higher concentrations and a larger range (3–11%) of the mixed-linked, nonstarch polysaccharide β-D-glucans (β-glucan). These nonstarch polysaccharides, present almost entirely in endosperm cell walls, have been partially purified from oats at a small scale (Wood et al 1978, Welch et al 1988) and more recently from defatted oat bran at a pilot-plant scale (Wood et al 1989), and their fine structure has been investigated (Wood et al 1991a). Isolated β-glucan may be used for conducting nutritional studies and for investigating functionality in food applications, industrial hydrocolloids, and pharmaceuticals. Cowan and Mollgaard (1988) described a pilot-plant procedure for the preparation of hydrolyzed β-glucan (after purification) for use as a bulking agent to replace sucrose.

Barley is a potentially useful grain for enrichment of β-glucan. Hull-less cultivars that eliminate dehulling and hull disposal are available. Hull-less barley may be dry-milled and easily separated into flour and bran fractions using conventional wheat-milling equipment (Bhatty 1986). Although barley has not traditionally been roller-milled like other cereals to obtain flour and bran, this may change in the near future because of barley's high soluble fiber and its potential use in many food products. Roller-milled barley bran thus may soon become commercially available. Barley bran contains one half of the oil of oat bran, and the oil need not be removed before β-glucan extraction, unlike oat bran oil.

This article reports the use of different solvents for the extraction and enrichment of β-glucan from low- and high-β-glucan barley brans and, for comparison, from commercial oat bran. The composition and some properties of the purified barley β-glucan preparations are reported as well.

MATERIALS AND METHODS

Materials

Tupper (six-rowed) registered Canadian hull-less barley containing low β-glucan (4.5%) was of our own stock grown at the University of Saskatchewan, Saskatoon, North Seed Farm, in 1988. Azhul (high β-glucan, 11.3%), a six-rowed waxy hull-less barley, was a gift from W. Newman of Montana State University, Bozeman. Oat bran was a commercial product obtained locally from RobinHood Multifoods, Saskatoon (variety unknown). Tupper and Azhul barleys were dry-milled to obtain bran in about 30% yields, using a short-flow milling procedure in an Allis-Chalmers experimental mill (Allis-Chalmers, Milwaukee, WI) (Bhatty 1986). The barleys and oat bran were ground in a Udy cyclone mill (Udy, Fort Collins, CO) to pass through a 1.0-mm screen.

The assay kit for β-glucan determination and commercial β-glucan (about 77% purity) were obtained from Biocon US Ltd. (Lexington, KY). Termamyl 120 L (α-amylase) was from Novo Industrie A/S Copenhagen, Denmark. TSK-Gel (Toyopearl), HW-65, particle size 30 to 60 μm, fractionation range 10^4 to 10^7 Da, was purchased from Supelco, Bellefonte, PA.

Extraction and Purification of β-glucan

Tupper barley bran and oat bran were extracted with solvent 1 only. Azhul barley bran was extracted with solvent 1 and with two more solvents. Solvent 1 was distilled water adjusted to pH 10 with 20% sodium carbonate. The ratio of bran to solvent was 1:10, and extraction time was 30 min at 45°C (Wood et al 1989). Solvent 2 was distilled water adjusted to pH 7, and the ratio of bran to solvent was 1:10. The extraction was performed sequentially at 40, 65, and 95°C, and then the three supernatant fractions were combined (McCleary 1988). Solvent 3 was 4% sodium hydroxide solution. The ratio of bran to solvent was 1:50, and extraction time was 18 hr at room temperature (Carr et al 1990). The rest of the procedure used for purification of the extracts was identical in each case (Fig. 1).

Analysis

Total β-glucan (brans and the freeze-dried preparations), extractable β-glucan (extracts), and nonextractable β-glucan (residues) were determined by the procedure of McCleary and Glennie-Holmes (1985). Acid extract viscosity, pentosans ([arabinose + xylose] × 0.88), and monosaccharides (by gas-liquid chromatography) were determined as described by Bhatty et al (1991). Starch analysis was performed by the procedure of Fleming and Reichert (1980); total nitrogen, ether extract, and ash were determined by using AOAC Official Methods (1990). For column chromatography, the TSK-Gel was prepared in 100 m*M* sodium acetate buffer, pH 5.4, according to the manufacturer's instructions and packed into a glass column (100 × 1.6 cm; total volume, about 200 ml). Void volume (75 ml) and elution volume (128 ml) were determined with blue dextran (Sigma; average molecular weight, 2×10^6). About equal concentrations of the purified β-glucan preparations and commercial β-glucan were dissolved in the acetate buffer by heating (<70°C) and were eluted from the column with the buffer at a flow rate of about 15 ml/min. Lichenase (0.2 ml) was added to 1.0-ml aliquots of the fractions, and the assay mixture was incubated for 1 hr at 40°C. To a 100-μl aliquot of the mixture, 0.2 ml of β-glucosidase was added and incubated at 40°C for 20 min. The glucose released was measured at 510 nm, using glucose oxidase-peroxidase reagent (McCleary and Glennie-Holmes 1985).

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RESULTS AND DISCUSSION

Several solvents have been reported in the literature for the extraction of β-glucan from barley, more often for its quantitative analytical determination without interference from contaminating starch (Martin and Bamforth 1981, Ahluwalia and Ellis 1984, Carr et al 1990) than for its enrichment. β-Glucan has been extracted from oat flour with sodium carbonate solution and purified for analytical and nutritional purposes (Wood et al 1978, Welch et al 1988), at a large preparative scale from oat bran (Wood et al 1989) and, more recently, from barley and oats by laboratory-scale dry milling and sieving (Knuckles et al 1992). The latter procedure yielded β-glucan-enriched (up to 28%) fractions in yields of about one-third of the original sample weight. Dry milling and sieving offers an advantage over wet extraction, where solvent disposal may be a problem. However, considerable starch (42–60%) and other components (not reported) present in the preparations may not be acceptable in some β-glucan applications. Wood et al (1978) investigated conditions for the extraction of β-glucan from oats and oat bran. These were used in this study without modification. However, barley and oat brans contain different levels of β-glucan and other components. Their solubility may differ in different solvents. For this reason, barley brans containing low or high β-glucan and, for comparison, oat bran were extracted with a 20% sodium carbonate solution, and Azhul barley bran was extracted with two additional solvents.

In a preliminary experiment, Tupper barley bran (6.6% β-glucan) and commercial oat bran (6.9% β-glucan) were extracted in 5-g quantities with solvent 1, and proportions of the extractable and nonextractable β-glucans were calculated as percentage of the total β-glucan. In barley bran, about 70% of the β-glucan was extracted, whereas in oat bran the extractability was 81% (Table I). The rest was nonextractable β-glucan. Reasons for the lower extractability of barley bran β-glucan may be many: differences in bran particle size, different size distribution of β-glucan, different or tighter cross-linking of barley β-glucan in the cell walls (Forrest and Wainwright 1977), and cultivar, stage of kernel development, and growing conditions that affect the content and solubility (water) of β-glucans (Åman et al 1989). Extractability of β-glucan from oat bran, on the basis of triplicate determination, had variability similar to extractability of barley bran β-glucan. In both cases, the coefficient of variation was about 8%. Although at this stage solvents were not compared for extractability of barley or oat bran β-glucan, subsequently it was found (Table II, solvent 3) that sodium hydroxide considerably improved the extractability of β-glucan from barley bran.

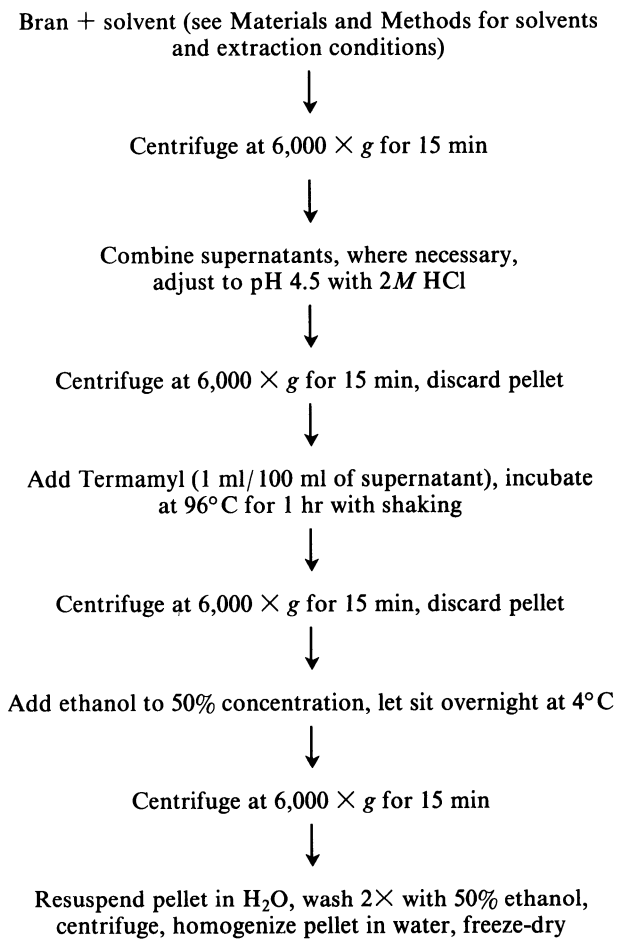

Fig. 1. Flow diagram of the β-glucan purification procedure.

In the next experiment barley and oat brans were extracted in 5- or 40-g quantities to determine mass balance of β-glucan and its recovery from the extractable fraction by following the purification procedure outlined in Figure 1. In barley brans, the extractable β-glucan was 61 and 63% for solvent 1, 72% for solvent 2, and 84% for solvent 3 (Table II). With oat bran, solvent 1 extracted 70% of the β-glucan, which was lower than the average obtained for the same solvent in Table I (80%). Extractability of β-glucan is critical, as it will determine the final yield of the preparation. Sodium hydroxide (solvent 3) extracted more β-glucan from Azhul barley bran (84%) compared with extraction from the same bran with solvent 1 (61%) or solvent 2 (72%). The residue, in each case vacuum-dried overnight at 60°C, was used to determine nonextractable β-glucan. The two fractions (extractable and nonextractable) accounted for 96–98% of the total β-glucan for solvent 1, 95% for solvent 2, 100% for solvent 3, and 90% for solvent 1 in oats. Table II also gives the final recoveries (yields) of β-glucan from the extractable fractions. The highest recovery was obtained with solvent 3 for a final β-glucan yield of 81% of that present in Azhul barley bran. With other solvents the recoveries were lower and variable: solvent 2 had the lowest (40%) followed by solvent 1 (52–61%) in barley and oat brans. Thus, yield differences were greater for the extraction solvents in Azhul barley bran than for solvent 1 in different brans. The purification procedure used in this study included treatment of the extract, after precipitation of protein, with Termamyl to remove contaminating starch. Treatment with an amylolytic enzyme seemed necessary in our experience as one preparation of β-glucan from Tupper bran (solvent 1) without treatment contained 14% starch, which was reduced to less than 1% on enzyme treatment.

Table III gives the comparative compositions of the β-glucan preparations. All of the preparations contained less than 1% total nitrogen, 0.4–1.2% starch, 3.7–12.6% ash, and 1.1–10.9% pentosan; ether extract was not detected in any of the preparations. The variable and high ash content of the preparations was most likely sodium chloride, which was easily washed out with 50%

TABLE I
Repeatability of β-Glucan Extraction from Barley and Oat Brans[a]

	Barley Bran (6.6% β-glucan		Oat Bran (6.9% β-glucan)	
	Extractable[b]	Non-extractable[c]	Extractable	Non-extractable
	67.6	32.4	75.1	24.9
	70.5	29.5	87.7	12.3
	62.2	37.8	78.0	22.0
	71.0	29.0		
	76.9	23.1		
Mean	69.6	30.4	80.3	19.7
Standard deviation	5.4	5.4	6.6	6.6
Coefficient of variance	7.8	17.8	8.2	33.5

[a]Shown as percentage of total β-glucan.
[b]Solvent 1 (20% sodium carbonate, pH 10), see Materials and Methods for details.
[c]Determined on oven-dried residue; extractable and nonextractable β-glucan are adjusted to total 100%.

ethanol. One β-glucan preparation from Azhul barley bran, extracted with sodium hydroxide (solvent 3), was dialyzed overnight against distilled water. This reduced the ash content from 12.6 to 3.3%. However, washing of the preparations with 50% ethanol before freeze-drying was preferable. The other highly variable component was the pentosans, undoubtedly because of their variability in the brans and also because of differences in solubility in different solvents. The β-glucan preparations from barley brans contained more pentosans than the preparation from oat bran. The solvent effect was even greater. β-Glucan preparations from Azhul barley bran extracted with solvent 3 contained 10.9% pentosans, compared with 1.9% for the same bran extracted with solvent 2 and 2.9% extracted with solvent 1. This was not surprising as sodium hydroxide is a common solvent for extraction of hemicelluloses or pentosans from cereals. The differences in ash and pentosan contents of the preparations affected their β-glucan concentration or purity. Therefore, for a better comparison of β-glucan and pentosan concentrations, both of which affect viscosity, data were reported as the sum of β-glucan and pentosans expressed on ash-free basis. On this basis, Tupper bran preparation contained 73%, Azhul barley bran preparations 78–82%, and oat bran preparation 84% β-glucan plus pentosans. Thus, extraction of Azhul barley bran with solvents 2 and 3 gave β-glucan preparations with a generally similar purity (81–82%) as that obtained from oat bran extracted with solvent 1 (84%). However, purity of the preparation needs to be evaluated in conjunction with yield or percentage of the total β-glucan recovered. The latter values are reported in Table II. These two expressions were taken to calculate an "enrichment index": yield × (concentration of β-glucan + pentosans, ash-free basis)/100. This expression, given at the bottom row of Table II, showed solvent 3 to be the better solvent for enrichment of β-glucan. Its enrichment index was 65, compared with values of 33 to 43 obtained for the other solvents used for barley brans and 51 for solvent 1 used for oat bran (Table II).

The major monosaccharide of the preparations was, as expected, glucose derived largely from β-glucan and to a minor extent from the contaminating starch (Table III). Azhul barley bran extracted with solvent 1 contained, for unknown reason, less glucose. Arabinose, galactose, and xylose were the other sugars. Fucose and rhamnose were present only in minute quantities. Arabinose and xylose were the next major sugars; the sum of these two in β-glucan preparation obtained with solvent 3 was four to six times higher than in the preparations obtained from the same bran extracted with solvents 1 and 2.

The three β-glucan preparations from Azhul barley bran extracted with solvents 1–3 were fractionated by size-exclusion chromatography (Fig. 2) to compare their apparent molecular weights with that of the commercial β-glucan. All four preparations eluted largely between the void volume (75 ml) and the elution volume (128 ml) of blue dextran, suggesting apparent average molecular weights approximately in the region of 2×10^6. Molecular weight of barley β-glucan varies with cultivar. Wood et al (1991b) reported a range of 1.70×10^6 to 2.66×10^6 for four preparations of barley β-glucans, forming 38–54% of the total β-glucan. β-Glucans from oat cultivars and brans had higher molecular weights (3.00×10^6). However, it is not easy to assign molecular weights to cereal β-glucans because of different extraction procedures that are further complicated by varietal and environmental influences. The β-glucan preparations extracted with solvents 2 and 3 eluted in a similar manner, and their molecular weights seemed somewhat larger than that of the commercial β-glucan, which in turn had larger molecular weight than the solvent 1 preparation. Solvent 1 preparation was more

TABLE II
Mass Balance of β-Glucan Extracted from Barley and Oat Brans (5 or 40 g) with Different Solvents[a]

	Tupper Bran, Solvent 1		Azhul Bran						Oat Bran, Solvent 1	
			Solvent 1		Solvent 2		Solvent 3			
β-Glucan	(mg)	(%)	(mg)	(%)	(mg)	(%)	(mg)	(%)	(mg)	(%)
Total	2,632	100	5,232	100	671	100	671	100	2,776	100
Extractable	1,670	63	3,211	61	486	72	566	84	1,932	70
Nonextractable	914	35	1,803	35	152	23	105	16	544	20
Recovered	2,584	98	5,014	96	638	95	671	100	2,476	90
Recovered from extractable	1,367	82	2,850	89	265	55	545	96	1,692	88
Percent total recovered (yield)		52		55		40		81		61
Enrichment index[b]		38		43		33		65		51

[a]Single extraction and purification from each bran with each solvent. Tupper bran, 6.6% β-glucan; Azhul bran, 13.4% β-glucan; oat bran, 6.9% β-glucan.
[b]Defined as yield × (concentration of β-glucan + pentosans on ash-free basis)/100.

TABLE III
Composition of β-Glucan Preparations Isolated from Barley and Oat Brans[a] (%, as is)

	Tupper Bran,	Azhul Bran			Oat Bran,
Component	Solvent 1	Solvent 1	Solvent 2	Solvent 3	Solvent 1
β-Glucan	57.9 ± 1.0	71.9 ± 0.4	74.9 ± 2.4	59.7 ± 0.3	79.7 ± 0.8
Nitrogen	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	0.5 ± 0.0
Starch	1.1 ± 0.1	0.4 ± 0.0	1.0 ± 0.1	0.4 ± 0.0	1.2 ± 0.0
Ether extract	0	0	0	0	0
Ash	9.5 ± 0.3	3.9 ± 0.6	6.8 ± 0.2	12.6 ± 0.2	3.7 ± 0.3
Pentosans	7.8 ± 0.3	2.9 ± 0.0	1.9 ± 0.1	10.9 ± 0.0	1.1 ± 0.0
β-Glucan + pentosans[b]	65.7 (72.6)	74.8 (77.8)	76.8 (82.4)	70.6 (80.8)	80.8 (83.9)
Monosaccharides					
Arabinose	3.4 ± 0.2	1.2 ± 0.0	0.7 ± 0.0	4.5 ± 0.1	0.5 ± 00
Fucose	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0
Galactose	1.1 ± 0.1	0.4 ± 0.1	1.9 ± 0.1	2.2 ± 0.1	1.2 ± 0.3
Glucose	49.3 ± 3.5	47.9 ± 0.5	54.0 ± 0.5	62.2 ± 1.2	61.3 ± 0.1
Rhamnose	0	0	0.2 ± 0.0	0.1 ± 0.0	0
Xylose	5.4 ± 0.3	2.1 ± 0.1	1.5 ± 0.0	8.0 ± 0.1	0.8 ± 0.0

[a]Data are means of duplicate determination (means and standard deviations).
[b]Figures in parentheses are on ash-free basis.

heterogenous than the other two preparations. Although equal concentrations of β-glucan for the three preparations were applied to the column, the peak height of solvent 1 preparation was lower and asymmetrical.

Although β-glucan extracted with solvents 2 and 3 seemed to have higher molecular weights than that extracted with solvent 1 (Fig. 2), the same relationship was not apparent in their acid extract viscosity (Fig. 3). The reverse seemed to be the case for the solvent 1 preparation, which had the highest flow viscosity. The preparations of solvents 2 and 3 had similar viscosity. The relationship between sample molecular weight and acid extract viscosity is tenuous, since molecular weight and structure affect flow properties of β-glucan (Wood et al 1991b). Nevertheless, viscosity is one of the most important properties of β-glucan for application in the food industry. In addition to its flow behavior, viscosity of β-glucan influences consistency and heat stability of food preparations (Autio et al 1987). These properties

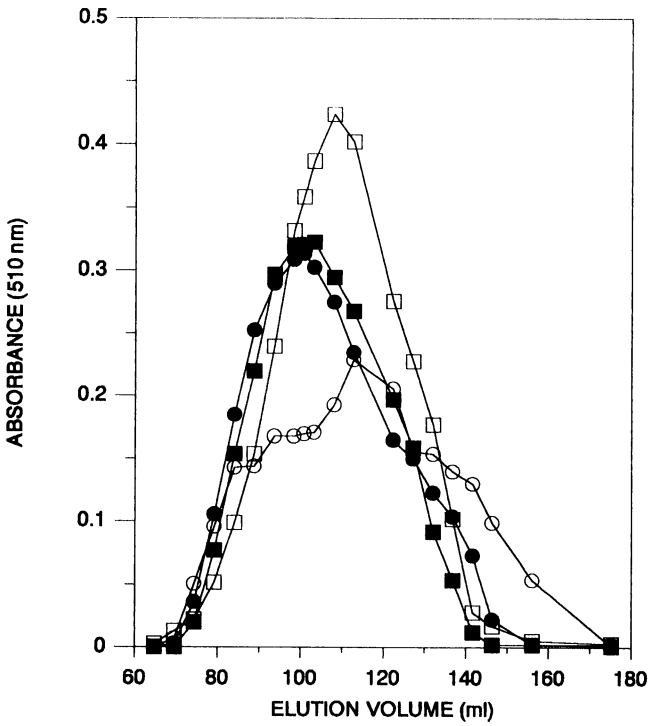

Fig. 2. Size-exclusion chromatography of commercial β-glucan (□) and β-glucan preparations obtained from Azhul barley bran with solvents 1 (○), 2 (●), and 3 (■). Blue dextran void volume was 75 ml and elution volume 128 ml.

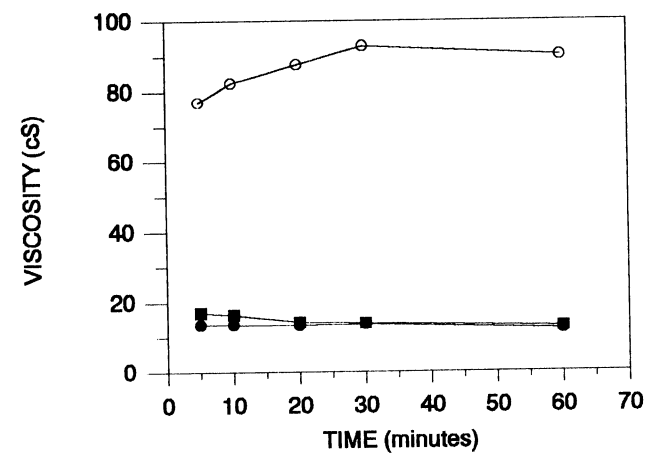

Fig. 3. Flow viscosity of β-glucan preparations extracted from Azhul barley bran with solvents 1 (○), 2 (●), and 3 (■). In each case, 5 mg/ml of β-glucan was used. See Materials and Methods for measurement of acid extract viscosity.

were reported for oat β-glucan, and they may be equally applicable to barley β-glucan in view of their similarity of structures and flow behavior. The extraction solvent appeared to affect viscosity; therefore, this parameter should be considered when selecting solvent for extraction and purification of β-glucan from barley bran.

CONCLUSIONS

Of the three solvents compared for β-glucan extraction and purification from barley brans, sodium hydroxide (solvent 3) seemed to be most efficient. A single extraction for 18 hr at room temperature at a ratio of meal to solvent of 1:50 extracted 80–85% of the bran β-glucan. Subsequent experiments (not given) showed that the ratio of meal to solvent could be reduced to 1:20 and the extraction time to 6 hr without affecting the extractability of the β-glucan; a second extraction under these conditions removed an additional 4% of the bran β-glucan. The final preparation obtained with this solvent contained generally the same concentration of β-glucan (and pentosans) expressed on ash-free basis (81%) as that obtained from oats with solvent 1 (Table III). The β-glucan yield or percentage of total recovered with this solvent from Azhul barley bran was 81% and its enrichment index 65, which considered yield and purity of the preparations, both being the highest compared with similar values obtained with the other two solvents used for extraction and purification of β-glucan from barley or oat bran (Table II). In addition, sodium hydroxide extracted more pentosans than other solvents. These nonstarch polysaccharides enhance the viscometric properties of β-glucan, a desirable feature in food applications.

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Influence of Gluten Components and Flour Lipids on Soft White Wheat Quality[1]

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ABSTRACT

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Soft white wheat flours from varied growing conditions were analyzed for selected variables of gluten and components of flour lipid to identify those that are associated with baking quality as measured by cookie diameter and cake volume. Statistical analysis indicated that among the gluten variables, yield of gluten and pentosan in gluten were the variables most associated with cookie diameter corrected for protein content. However, when the correction for protein content was not taken into account, total protein was shown to be negatively correlated with cookie diameter. Among the components of flour lipid, polar lipid had the highest correlation with cake volume. These variables, therefore, appear to be important in the end-use quality of soft white wheat.

Soft wheat flours with low protein content are used for pastry products rather than for breadmaking, where hard wheat flour with higher protein content is used (Hoseney et al 1988). However, the protein content is not the only factor determining end-use properties. Protein quality can influence the baking properties of both hard wheat flour (Orth and Bushuk 1972) and soft white wheat flour (Kaldy and Rubenthaler 1987).

An important factor in protein quality is the gluten characteristics of a dough. Gluten is formed by the interactions of the proteins, glutenin and gliadin, which also associate with lipid and pentosans during dough formation (D'Appolonia and Kim 1976, Hoseney 1986). A strong dough with an extensive gluten network is suitable for breadmaking (Pomeranz 1988). In contrast, a weak dough, without an extensive gluten network, is best for cookies and cakes (Gaines 1990). Consequently, flour quality is influenced by the nature of the gluten and its various components. However, very little is known about gluten and its components in soft white wheat flour, especially with reference to baking quality.

Pentosans also have an influence on flour quality and dough formation (Yamazaki 1955, Shogren et al 1987). Native flour pentosans were found to have a negative effect on both cookie diameter and cake volume (Kaldy et al 1991). Some pentosans are part of the gluten matrix, but the exact nature of the association with the matrix is unknown. Also, the influence of the gluten-associated pentosans on baking quality of soft white wheat flour has not been established.

Flour lipids also influence the baking quality of soft white wheat flour. Cookies baked from flours with the lipids removed had smaller diameters than those baked from unextracted flours (Cole et al 1960). Studies on cake quality have also indicated that removal of lipids reduces cake volume (Seguchi and Matsuki 1977).

As far as lipid fractions are concerned, nonpolar and polar lipids in spring wheat flour have been shown to have an influence on loaf volume (Bekes et al 1986). Both nonpolar and polar lipids were found to be necessary for the restoration of cookie spread in defatted flour from a cultivar of eastern soft white wheat (Kissell et al 1971).

The purpose of this study was to examine gluten and lipid components in flours from soft white wheat grown under a broad range of conditions and to identify the gluten and lipid variables most associated with baking quality.

MATERIALS AND METHODS

Soft white wheat samples were collected from a broad range of growing environments in Alberta and Ontario, Canada, and in Washington State. Fifteen samples of soft white spring wheat were from a 500-km-wide region of Alberta. Ten samples were cv. Fielder, one was cv. Owens, one was cv. Dirkwin, and the remaining three samples were mixed (commercial) spring wheat cultivars, mainly Fielder and Owens. All were grown in 1984 except one Fielder sample, which was grown in 1983. Of the five samples from Washington State, four were Fielder. One was grown in 1981, another in 1982, and two samples in 1983. The other sample was Owens, grown in 1984. Three of the five winter wheat samples from Ontario were the cultivar Fredrick, grown in 1982, 1983, and 1984. The two other samples were mixed (commercial) cultivars grown in 1984.

All samples were milled on a Buhler pneumatic laboratory mill at the USDA Western Wheat Quality Laboratory, Pullman, WA, as described previously (Kaldy and Rubenthaler 1987). Cookie and cake baking, as well as some of the other tests, were also done at the Western Wheat Quality Laboratory as described previously (Kaldy and Rubenthaler 1987). The samples were stored under refrigeration. Some of the carbohydrates in flour were analyzed as previously described (Kaldy et al 1991).

As reported previously (Kaldy and Rubenthaler 1987, Kaldy

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