Enzymic Determination of β-Glucan in Cereal-Based Food Products

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ABSTRACT

An improved method was developed for the determination of (1→3), (1→4)-β-D-glucans in cereal-based food products and cereal grains. The method uses refluxing 80% (v/v) ethanol to remove sugars and inactivate enzymes prior to 1-hr extraction with water at 100°C for soluble β-glucan determination or 16-hr extraction with 1.0*N* NaOH for total β-glucan determination. An enzyme preparation from *Penicillium funiculosum* is used to selectively and quantitatively liberate glucose from β-glucan in the extracts. For several different food products, soluble glucan content ranged from 0.49 to 3.90%, whereas total β-glucan content ranged from 0.58 to 8.86% (dry weight basis). Total dietary fiber ranged from 4.8 to 22.0% for the products. Total β-glucan content is also reported for six different cereal grains. The preextraction with refluxing 80% ethanol is not required for the unprocessed cereal grains.

Cereal grains contain various levels of (1→3), (1→4)-β-D-glucans, hereafter referred to as β-glucans. This polysaccharide is ubiquitous among the cereal grains but occurs in highest amounts in the endosperm cell walls of barley and endosperm and bran of oats (Henry 1987, Wood 1984). Barley β-glucans have been studied extensively within the brewing industry and in animal feeding studies. In the brewing industry, barley β-glucans are responsible for retardation of malting, poor wort separation, difficulties in beer filtration, and formation of undesirable beer precipitates (Bamforth 1982). In animal feeds, barleys fed to monogastric animals may cause poor growth performance, in part due to β-glucans in the barley grain (Burnett 1966, White et al 1981, Newman et al 1987).

The role of cereal β-glucans in human nutrition and health has recently been reviewed (Klopfenstein 1988). Oat products as a source of soluble dietary fiber have recently become a key focus of the nutrition and medical communities. Rich in water-soluble fiber, oat products have been shown to exhibit distinct hypocholesterolemic effects in humans (Anderson et al 1984), and the major cholesterol-lowering component of oat products appears to be the water-soluble gum (Chen et al 1981). A similar hypocholesterolemic effect has been shown for barley products in both humans (Newman et al 1987) and chicks (Fadel et al 1987).

An increasing number of studies have examined the role of soluble fiber components in human health, but the contribution of specific complex carbohydrates has not been systematically investigated. Research on specific dietary fiber components such as pectin, cellulose, and lignin has been facilitated by the availability of methods to analyze for these components in food products (Jeltema and Zabik 1980, Olson et al 1987). Nutritional and physiological studies of β-glucans in foods, however, have been limited by the lack of a simple method for quantitative analysis.

With the numerous reports that oat products are good sources of soluble dietary fiber, food manufacturers have responded with a proliferation of oat-based products for the marketplace along with other fiber-supplemented food products. Because these fiber-supplemented products vary greatly in the types of cereal and noncereal fibers present and in the types of processing employed in their manufacture, a method specific for the determination of β-glucan in the finished food product is necessary.

It is of interest to identify the contribution of individual food components to dietary fiber in the diet. The addition of β-glucan fractions from oats, barley, wheat, and sorghum to experimental white pan breads was recently examined (Klopfenstein and Hoseney 1987). Data on actual β-glucan content by direct analysis of the added β-glucan fractions or the experimental breads used in the feeding studies were not cited. The need for an accurate β-glucan analytical method for cereal-based food products is apparent as an increasing number of studies investigate the role of specific dietary fiber components.

Various extraction and quantitation techniques have been suggested for the determination of β-glucan in cereal grains. Problems with these methods often include incomplete extraction or hydrolysis of β-glucans, lack of pure β-glucanases, and unavailability of good reference standards for calibration of rapid spectrofluorometric and viscosity-based methods (Wood and Weisz 1984, Wood et al 1987). The techniques most commonly employed in the brewing industry and by plant breeders are those based on a purified β-glucanase from *Bacillus subtilis* (McCleary and Glennie-Holmes 1985) and fluorometric methods that use

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the optical brightener, Calcofluor (Wood and Weisz 1984). An automatic flow injection analysis system has been developed based on complex formation between β-glucan and Calcofluor (Jørgensen 1988, Jørgensen and Aastrup 1988a). Another method that uses a β-glucanase from *Penicillium funiculosum* has also been proposed for the determination of barley β-glucan (Bamforth 1983). These methods and others are described in a recent review (Jørgensen and Aastrup 1988b).

The objective of the research reported in this paper was to develop a method for determination of both water-soluble and total β-glucan in cereal-based food products that would overcome potential errors associated with soluble sugars and endogenous enzyme activities often present in food samples. This method, based on the β-glucanase from *P. funiculosum*, was then employed in the characterization of several commercially available products, many of which are considered good sources of dietary fiber and, more specifically, soluble oat fiber.

MATERIALS AND METHODS

Sample Preparation and Extraction

Test products were purchased locally and represent national brands and in-store bakery products. The air-dried food products were ground to pass a 20-mesh sieve and then stored at −20°C in polyethylene bottles until analyses were performed. Samples were dried in vacuo (55°C, 20 hr), weighed (200 mg), and extracted two times with refluxing 80% (v/v) ethanol (5 ml) for 0.5 hr each. The extracted residues were recovered quantitatively after cooling and the aqueous ethanol extracts were discarded.

Water-soluble β-glucan was extracted from the ethanol-treated residues with water (10 ml) in a shaking water bath at 100°C for 1 hr. The extracts were centrifuged to remove insoluble material and the supernatants were collected. The residues were washed with an additional 10 ml of water and the combined supernatants were then pooled and adjusted to volume before aliquoting.

Total β-glucan content was determined similarly by extracting the ethanol-treated residues with 1.0*N* NaOH (10 ml) at 20°C for 16 hr. The extraction mixtures were neutralized with 1.0*N* HCl, centrifuged to remove insoluble material, and the supernatants were collected. The residues were washed with an additional 10 ml of water and the extracts and washings were pooled and adjusted to volume before aliquoting.

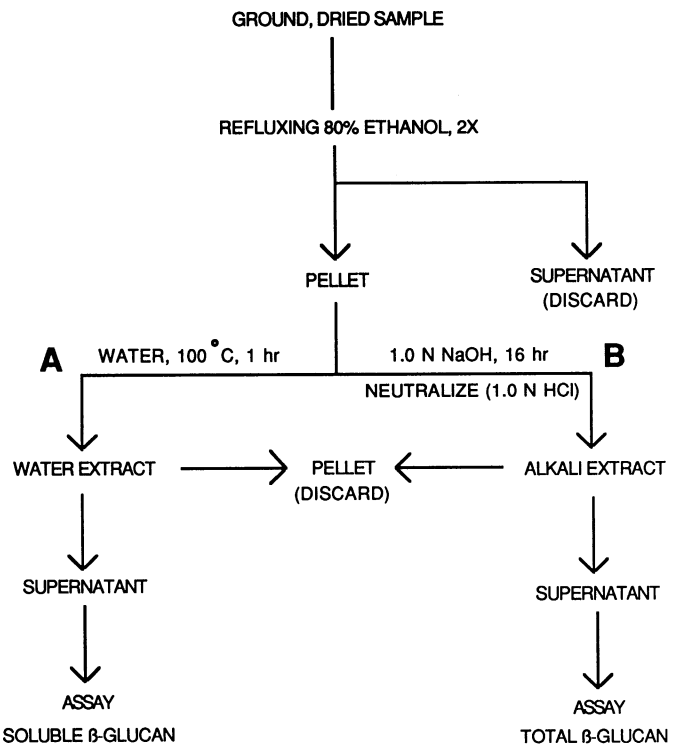

Fig. 1. Quantitative extraction procedure for soluble (**A**) and total (**B**) β-glucan determination in cereal-based food products.

β-Glucan Assay

The assay uses a commercial cellulase (Sigma C 0901) from *P. funiculosum*, heat-treated to remove contaminating amylolytic activity as previously described (Bamforth 1983). The crude enzyme (0.40 g) was suspended in 0.05*M* sodium acetate-HCl buffer (pH 4.0, 10 ml) and after 10 min centrifuged at 2,500 rpm for 10 min. The supernatant was transferred to 5 tubes (1 × 10 cm) and heated at 70°C in a water bath for 1 hr, with subsequent cooling in an ice bath for 2 min. The supernatant was dialyzed against 2 L of 0.05*M* sodium acetate-HCl buffer at 4°C for 16 hr and then centrifuged for 30 min. The supernatant was collected and stored at 4°C for up to one week. For each week of storage, this enzyme preparation lost 15–20% of its β-glucan hydrolyzing activity and gave increasing blank values. However, the heat-treated preparation was lyophilized and stored at −20°C with no appreciable loss of activity for at least eight months.

Triplicate sample aliquots (0.40 ml) were transferred to tubes containing 0.05*M* sodium succinate buffer (pH 5.5, containing 0.02% sodium azide) (0.40 ml) and enzyme solution (0.20 ml). The sample tubes and appropriate enzyme and sample blanks were incubated at 40°C for 3 hr prior to determination of liberated glucose by the glucose oxidase-peroxidase procedure (Karkalas 1985). Each heat-treated enzyme batch was tested for contaminating amylolytic activity and adequate β-glucanase activity by incubating enzyme samples under standard conditions with starch and barley β-glucan (Biocon, Inc.), respectively. Control samples of starch and β-glucan were carried through the assay along with the samples being analyzed.

Dietary Fiber Assay

Total dietary fiber (TDF) content was analyzed by the modified AOAC method (Prosky et al 1985, 1986). All results were corrected

TABLE I
Comparison of Different Extractants for Assay of Total β-Glucan in Cereal Grains

Grain Sample	Variety	Total β-Glucan Extracted (% dry weight)[a]			
		Urea[b]	Acid[c]	Alkali[d]	Alkali + $NaBH_4$[e]
Oats	Centennial	2.65 (0.17)	2.48 (0.25)	6.63 (0.10)	6.59 (0.10)
Barley	Klages	2.99 (0.12)	2.23 (0.06)	4.89 (0.15)	4.43 (0.10)
Triticale	Lasko	0.23 (0.01)	0.35 (0.03)	0.40 (0.01)	nd[f]
Rye	Unknown	0.37 (0.04)	1.03 (0.02)	1.88 (0.06)	nd
Wheat	Unknown	0.05 (0.01)	0.69 (0.02)	0.81 (0.02)	nd

[a]Values are means for three replicates (standard deviation).
[b]7*M* urea (25°C, 16 hr).
[c]Perchloric acid (50 m*M*, 95°C, 3 min).
[d]1.0*N* alkali (25°C, 16 hr).
[e]1.0*N* alkali + 6 mg/ml $NaBH_4$ (25°C, 16 hr).
[f]Not determined.

for undigested protein (Kjeldahl N × 6.25) and ash (ignition at 525°C for 8 hr).

RESULTS AND DISCUSSION

The purpose of this study was to develop an improved enzymic assay for both water-soluble and total β-glucan in various cereal-based food products. In this study a heat-treated cellulase preparation, first suggested for determination of barley β-glucan (Bamforth 1983, Ahluwalia and Ellis 1984), was employed for assay of β-glucan. Several different lots of the commercial cellulase from *P. funiculosum* were examined, and they demonstrated consistent inactivation of amylolytic activity (less than 0.1% hydrolysis of starch to glucose) and adequate retention of β-glucanase activity (complete hydrolysis of β-glucan to glucose) for use of the enzyme preparation in routine β-glucan assay.

An extraction scheme for soluble and total β-glucan was developed based on boiling water and alkaline extraction, respectively (Fig. 1). This new method overcame inherent problems with products high in added sugars and/or confounding enzyme activities, particularly amylases. Direct extraction of certain cereal-based food products gave high blank values (up to 60% of measured glucose) because of the presence of sugars or other interfering substances. Removal of sugars from barley and malt samples by ethanol extraction or sodium borohydride reduction prior to enzymic hydrolysis has been suggested (Anderson et al 1978, Henry and Blakeney 1986). These methods were examined and the preferred method was found to be extraction with refluxing aqueous ethanol. This treatment removed interfering substances and inactivated endogenous or added enzymes, allowing accurate estimation of both soluble and total β-glucan in the samples.

There is no universally accepted definition for soluble β-glucan; instead, it is normally defined by the extraction conditions used in its isolation. For this study, soluble β-glucan was defined as that which is extractable in water at 100°C within a 1-hr extraction period. This extraction time was selected because the amount of β-glucan extracted from oat products was observed to vary during different cooking procedures (Yiu et al 1987). However, as soluble fiber components and their definitions are further examined, the conditions for extraction of "soluble" β-glucan in this assay may be modified in accordance with the evolving definitions.

Total β-glucan is, perhaps, more easily defined than soluble β-glucan. Different extractants including urea, acid, water, and alkali were examined for extraction of total β-glucan. The results for several different cereal grains showed that 1.0*N* NaOH provided the greatest yield and the most reproducible results (Table I). The small amount of β-glucan remaining in the residue after extraction under the assay conditions described does not lead to a significant underestimation of total β-glucan for the sample. Inclusion of sodium borohydride during extraction to prevent alkaline degradation of the β-glucan did not improve extraction yield (Table I). Therefore, it has not been included in the alkaline extraction step for routine analyses.

The quantity of β-glucan in the sample extractable by water at 100°C (defined as soluble β-glucan) and that extractable by 1.0*N* alkali (defined as total β-glucan) were determined for several cereal-based foods (Table II). Soluble β-glucan content ranged from 0.49 to 3.90%, and total β-glucan content ranged from 0.58 to 8.86% (dry weight basis). Oat bran hot cereal contained the highest β-glucan content (3.90% soluble and 8.86% total), and oat bran muffins had the lowest (0.49% soluble and 0.58% total). The products exhibited a wide variation in total β-glucan content and in the relative proportion of soluble β-glucan.

The magnitude of this variation in β-glucan content of these food products suggests that product labeling that includes β-glucan content would be of interest to the consumer in selecting breakfast cereals for their possible hypocholesterolemic action.

Total dietary fiber content is presented for the products in Table III. Product moisture, protein, ash, and manufacturer's recommended serving size are also presented to allow manipulation of β-glucan data presented in Table II.

The potential application of β-glucan as a food hydrocolloid because of its interesting rheological properties has been suggested. β-Glucan may be used as a thickening agent in sauces, salad dressings, or in ice cream manufacture (Wood 1986). If these products are pursued by the food industry, this β-glucan assay would be applicable.

The recent interest in the cholesterol-lowering effects of oat products and possibly other cereal-based foods led to the development of this assay. The assay can be used in specific

TABLE II
β-Glucan Composition of Various Cereal-Based Food Products (% dry weight)[a]

Food Product[b]	Soluble β-Glucan	Total β-Glucan
"Quick" rolled oats	2.45 ± 0.27	4.30 ± 0.12
Rolled oats	1.97 ± 0.07	4.61 ± 0.07
Oat RTE cereal	2.05 ± 0.11	2.48 ± 0.17
Oat bran RTE cereal	1.40 ± 0.11	1.90 ± 0.16
Oat bran flaked RTE cereal	1.63 ± 0.08	2.08 ± 0.12
Oat bran hot cereal	3.90 ± 0.29	8.86 ± 0.34
Oat bran	2.95 ± 0.23	7.28 ± 0.24
Granola bars	0.77 ± 0.08	1.42 ± 0.18
Oat bran muffins	0.49 ± 0.06	0.58 ± 0.09
Oatmeal bread manuf. A	0.86 ± 0.06	0.91 ± 0.06
Oatmeal bread manuf. B	1.00 ± 0.09	1.12 ± 0.05

[a]Soluble β-glucan is extracted with water at 100°C for 1 hr; total β-glucan is extracted with 1.0*N* alkali for 16 hr. Values are means for three replicates ± standard deviation.
[b]RTE designates ready-to-eat.

TABLE III
Composition of Various Cereal-Based Food Products (% dry weight)

Food Product[a]	TDF[b] (%)	Moisture (%)	Protein (%)	Ash (%)	Serving Size[c]
"Quick" rolled oats	11.3 ± 1.1	9.12	16.84	4.17	1 oz.
Rolled oats	11.5 ± 1.2	8.66	17.99	3.20	1 oz.
Oat RTE cereal	9.2 ± 0.5	1.86	14.08	2.63	1 oz.
Oat bran RTE cereal	18.2 ± 1.6	1.24	10.96	2.96	1 oz.
Oat bran flaked RTE cereal	11.2 ± 2.7	1.72	13.33	1.10	1 oz.
Oat bran hot cereal	21.6 ± 0.6	6.20	24.07	4.05	1 oz.
Oat bran	21.5 ± 1.2	10.50	22.85	3.20	1 oz.
Granola bars	8.9 ± 2.8	3.11	7.84	1.18	1 bar (28.5 g)
Oat bran muffins	6.1 ± 1.7	26.64	8.82	2.62	1 muffin (170 g)
Oatmeal bread manuf. A	6.0 ± 1.7	39.09	16.07	2.24	2 slices (68 g)
Oatmeal bread manuf. B	7.2 ± 1.1	37.95	17.46	3.52	1 slice (36 g)

[a]RTE designates ready-to-eat.
[b]Total dietary fiber values are means for three replicates ± standard deviation. Values are corrected for ash (525°C, for 8 hr) and protein (Kjeldahl × 6.25).
[c]Manufacturer's suggested serving size.

nutritional and physiological studies aimed at identifying the contribution of β-glucans to cholesterol-lowering effects.

Although the main emphasis of this paper is the application of this assay to processed cereal-based foods that frequently contain low molecular weight sugars (glucose and glucose-containing oligosaccharides), the method is also applicable to analysis of cereal grains. Table I gives the total β-glucan content for six cereal grains using the 1.0*N* NaOH extraction procedure. For these samples and a large number of other varieties of oats and barley, the sample blanks were sufficiently low so that the pre-extraction with refluxing aqueous ethanol was not required. Thus, the data in Table I were obtained directly on the alkaline extract of the ground whole grain, which simplifies the assay further. However, the hot aqueous ethanol extraction step should be used for any products containing levels of glucose or glucose syrups that would give high sample blanks.

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