Structure-Function Relationships of Alkaline Peroxide-Treated Lignocellulose from Wheat Straw

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ABSTRACT

Cereal Chem. 66(3):213-217

Compared to wood pulp cellulose, alkaline hydrogen peroxide treated wheat straw becomes more extensively hydrated, increases more in size when hydrated, and contains a larger glucose accessible internal volume. These and other data are interpreted in terms of a model for alkaline hydrogen peroxide treated lignocellulose in which the residual, peroxide-resistant lignin that remains after treatment functions as a skeletal framework, preserving an open internal structure within the lignocellulosic matrix, allowing greater water penetration and more thorough hydration of cell wall polysaccharides.

High-fiber ingredients can be incorporated in baked foods to reduce caloric density and increase dietary fiber content. The use of fiber ingredients such as native plant cell wall fractions (brans, vegetable pulps) or highly purified cellulose preparations (alpha-cellulose, wood pulp cellulose, microcrystalline cellulose) has been limited by the fact that these materials tend to degrade product quality, decrease baked volume, and introduce gritty texture (DeFouw et al 1982, Dubois 1978, Pomeranz et al 1976, Prentice and D'Appolonia 1977, Rajchel et al 1975, Satin et al 1978, Shogren et al 1981, Springsteen et al 1977, Titcomb and Juers 1986, Volpe and Lehmann 1977, Zabik et al 1977). It has been proposed that both of these effects are related to the inability of the cellulosic particles to absorb water, hydrate, and soften so that they become an integral part of the starch/gluten matrix, rather than functioning as particulate inclusions interrupting matrix continuity (Gould et al 1989).

Recently we reported that treating native cell wall fractions with dilute hydrogen peroxide at pH 11.5 markedly improved the ability of plant cell wall materials to become integrated into dough and batter-based products, even when substituted for up to 40% of the flour in the formulation (Jasberg et al 1989a,b). Compared to other fibers, alkaline hydrogen peroxide (AHP)-treated lignocelluloses improved baked volume and water-holding capacity of breads and cakes and increased dough tensile strength without introducing gritty textures or off-flavors.

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Treatment of lignocellulosic materials with AHP removes about half of the lignin (Gould 1984) and a variable portion of the hemicellulose (Gould 1985a) originally present in the cell walls, resulting in a disruption of the cell wall's morphological integrity (Gould 1985b) and an increase in the water-holding capacity of the substrate (Gould 1989, Gould et al 1989). Thus, the structure and composition of AHP-treated lignocellulose might be expected to fall somewhere between native (untreated) cell walls and highly purified cellulose preparations. In this paper we have examined, using wheat straw as a model system, some relationships between the physical properties of AHP-treated lignocellulose and its unique functionality in baked products.

MATERIALS AND METHODS

Alkaline Peroxide-Treated Lignocellulose

Wheat straw was chosen as a representative lignocellulosic substrate for the studies reported here because of the body of data already available concerning its functionality in baked systems (Jasberg et al 1989a, 1989b). The effects of AHP treatment were also documented on a variety of other materials more suited for use in human foods, such as cereal brans and fruit and vegetable pulps (Gould 1989, Gould et al 1989). Wheat straw was treated with AHP as described in detail elsewhere (Gould 1987). Dry, chopped (2–5 cm) straw was suspended in water (20–40 g/L) containing 10 g/L of hydrogen peroxide, and the slurry was adjusted to pH 11.5 with NaOH. The mixture was stirred gently for about 18 hr. The slurry was then neutralized to pH 6–7 with HCl, and the insoluble fraction was collected by filtration through a fine-meshed wire screen. The treated straw was washed thoroughly with water and then dried in a forced-air oven (Proctor Schwartz) at 40° C for 24 hr. For evaluation of water absorbency and swollen volume, the dried samples were ground in a Wiley mill (Arthur Thomas Co., Philadelphia, PA) using a 0.5- or 1-mm screen, as specified below. Alternatively, samples were ground in a pin mill (14,000 rpm, Alpine model 160Z, Augsburg, Germany). A portion of the pin-milled material was further ground in a ball mill (U.S. Stoneware Co., 19-L jar, 54 rpm, 25-mm granite balls, 40% charge, 1:1 material-to-void volume ratio) for 7 hr. For some experiments, treated wheat straw was partially dewatered after the washing step using a hydraulic press (Williams-White, Moline, IL) with a total force of 600 tons (approximately 100 kg/cm). The pressed material (approximately 50% solids) was then dried and ground as described above. Samples of never-dried material were also taken immediately after the washing step, and stored at 4° C until use. Wood pulp cellulose (Solka Floc SW-40, a pin-milled sulfite pulp) was obtained from James River Corp.

Determination of Water Absorbency and Swollen Volume

The capacity of sample materials to absorb water was estimated by mixing several grams of the dry sample with an excess of distilled, deionized water and allowing the sample to hydrate for 2 hr. The excess water was then removed by allowing the wet sample to drain on a fine-meshed wire screen. A portion of the wet sample on the screen was carefully removed, weighed, and then dried to constant weight in a forced-air oven (110° C). The water absorbency (in grams of water absorbed/gram dry material) for each sample was defined as (wet weight − dry weight)/dry weight. The volume occupied by the fully hydrated lignocellulosic samples (swollen volume) was measured by mixing 1 g of fiber with a large excess of distilled, deionized water in a graduated cylinder. The suspension was mixed intermittently for several hours to ensure complete hydration of the sample and allowed to settle overnight. The volume in the cylinder occupied by the swollen particles was taken as the swollen volume in milliliters per gram of dry fiber.

Particle Size Analysis

The particle size distribution of the wood pulp cellulose and alkaline peroxide-treated wheat straw (Wiley milled, 0.5-mm screen) was determined using 20.3-cm diameter U.S. Standard sieves. Screens were washed before each analysis and dried to constant weight in a forced-air oven at 105° C. For determination of dry particle size distribution, 10.0 g of dry material (<6% moisture) was placed on the sieve screen with the largest openings, and the screens were shaken on a Ro-Tap testing sieve shaker for 30 min. Four 1.2-cm, 1.3-g plastic balls were placed on each screen to increase agitation of the material and prevent matting of the fibers during separation. After shaking, the screens were disassembled and weighed to determine the amount of material retained on each screen. The particle size distribution of wet samples was determined in a similar fashion. Samples (25 g, dry weight) were allowed to hydrate for 30 min in a large excess of water. The suspended particles, in five approximately equal portions, were then slowly filtered through the set of sieve screens. Additional water was added continuously to the top screen to keep samples from matting on the screens. Water was gently added parallel to the screen surface so that fibers would not be forced through screen openings. When no more material could be observed passing through the top screen, the sieve was removed, allowed to drain, and the sample was dried to constant weight. Water was then added carefully, as described above, to the next screen, and the process repeated for each screen in the set.

Chromatography

Samples of alkaline peroxide-treated wheat straw (oven dried, Wiley milled, 0.5-mm screen) and the wood pulp cellulose were each fractionated according to particle size using the dry screening method described above. The fractions passing through a 60-mesh screen (0.250 mm opening) and retained on a 100-mesh screen (0.149 mm opening) were collected and hydrated in an excess of distilled water before being poured into 1.0-cm (i.d.) × 46 cm long chromatography columns. The column beds were washed for several hours using distilled water as eluent. The columns were then loaded with 0.5 ml of a solution containing native B-742-S dextran (Cote and Robyt 1983, average molecular weight [MW] $\geqslant 2 \times 10^7$) and glucose. The columns were eluted with distilled water by gravity flow, under an 0.8-m hydrostatic pressure head, and 1-ml fractions of the eluates were collected. Aliquots (100 μl) of each fraction were assayed colorimetrically (485 nm) for total carbohydrate using the phenol-sulfuric acid method (Dubois et al 1956).

RESULTS AND DISCUSSION

The ability of lignocellulosic materials to absorb water is markedly enhanced after these materials have been treated with AHP. This increase may be the result of at least two different effects: 1) an increase in the extent of hydration of cell wall components, especially polysaccharides, and 2) an increase in the amount of interstitial space within the cell wall particles. The contribution of the latter can be minimized by measuring the water content of cell wall material in which the interstitial volume has been greatly reduced by physically crushing the cell walls in a press. Under these conditions, most of the water remaining in the cell walls is assumed to be involved in direct hydration of cell wall components. This effect is illustrated by the data presented in Figure 1. Subjecting water-soaked wheat straw to increasing pressure using a hydraulic press reduced the amount of water associated with the straw until a plateau was reached, beyond which further increases in pressure had little effect. Wheat straw that had been treated with AHP retained significantly more water under high pressure than did the untreated straw, depending upon how the straw was handled after treatment. Peroxide-treated straw that was not dried after treatment retained twice as much water under pressure as untreated straw. Drying treated straw in a forced-air oven, followed by pin or ball milling, significantly reduced the ability of the straw to retain water under pressure. Straw that had been crushed by pressing in a hydraulic press after AHP treatment, dried in a forced-air oven, and ground in a ball mill exhibited an even greater reduction in its ability, when rehydrated, to retain water under pressure. Water retention by purified wood pulp cellulose was similar to that exhibited by pressure-dried, ball-milled peroxide-treated straw. Interestingly, the relative abilities of the peroxide-treated straw preparations to

retain moisture under pressure correlate with their relative effectiveness as a replacement for flour in bread doughs (Jasberg et al 1989a).

Previously we reported that fully hydrated AHP-treated lignocelluloses occupied a greater volume in water, on a dry weight basis, than untreated lignocelluloses (Gould et al 1989). The particle size distribution of a preparation of AHP-treated wheat straw under both dry and hydrated conditions is shown in Figure 2. The obvious shift in size distribution towards larger particles when the straw was fractionated wet demonstrates clearly that the individual straw particles increased in size when hydrated. Purified wood pulp cellulose exhibited a much smaller change in its particle size distribution when hydrated (Fig. 3).

The ability of AHP-treated wheat straw to absorb water and swell was dependent upon the size of the particles (Fig. 4). Particles retained (dry) on a screen with a 0.2–0.3-mm opening exhibited slightly greater water absorptions and swollen volumes than did larger particles. As particle size decreased below about 0.2 mm, both water absorption and swollen volume decreased rapidly.

AHP treatment selectively removes only about half of the lignin present in wheat straw and other lignocellulosics, but results in higher water absorption and greater functionality in baked foods than either untreated cell walls or completely delignified cellulose preparations (Jasberg et al 1989a,b). This suggests that the residual lignin in AHP-treated lignocellulose may function to maintain an open internal structure within the cell wall lignocellulose matrix. To determine the degree of porosity of AHP-treated lignocellulose, we compared the molecular exclusion characteristics of AHP-treated wheat straw with those of purified wood pulp cellulose. When a mixture of glucose (MW = 180) and dextran (MW = 2×10^7) was passed through a column of purified wood pulp cellulose, both compounds eluted simultaneously, in the excluded volume, indicating that both the low molecular weight glucose and the high molecular weight dextran were unable to enter internal regions of the cellular particles (Fig. 5). However, when the same experiment was performed using a column containing AHP-treated wheat straw, glucose eluted from the column after dextran (Fig. 6), indicating that glucose was able to penetrate into an internal region of the treated lignocellulose that was unavailable to dextran,

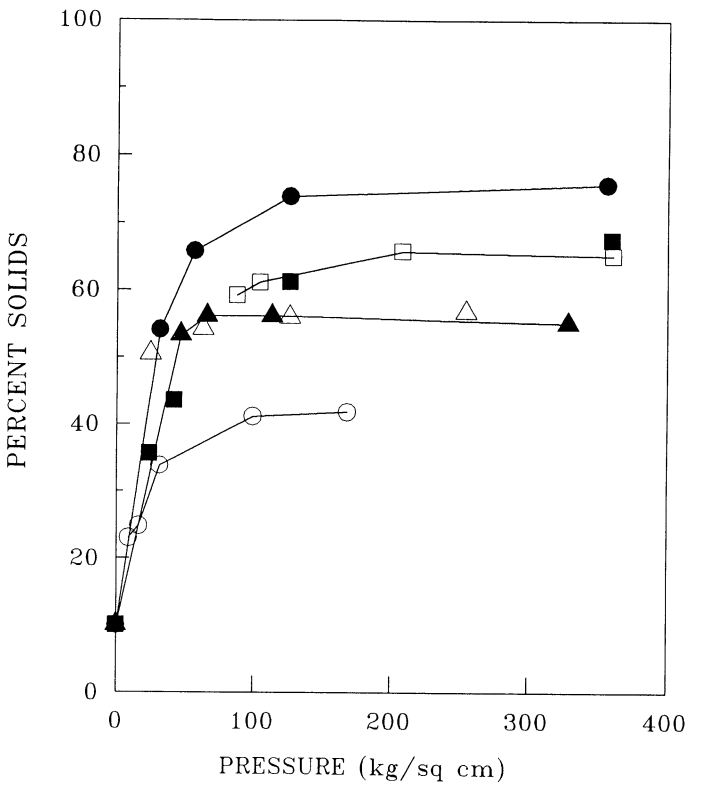

Fig. 1. Retention of water by various cellulosic materials under mechanical pressure. Aqueous suspensions of the various materials (10% solids) were hydrated and then subjected to mechanical pressure in a hydraulic press. The materials tested were: ● = wheat straw (1-mm Wiley milled); ▲ = alkaline hydrogen peroxide treated wheat straw (AHP-WS), pin milled; Δ = AHP-WS, ball milled; □ = AHP-WS, pressed, dried, and ball milled; o = AHP-WS, never dried; ■ = wood pulp cellulose.

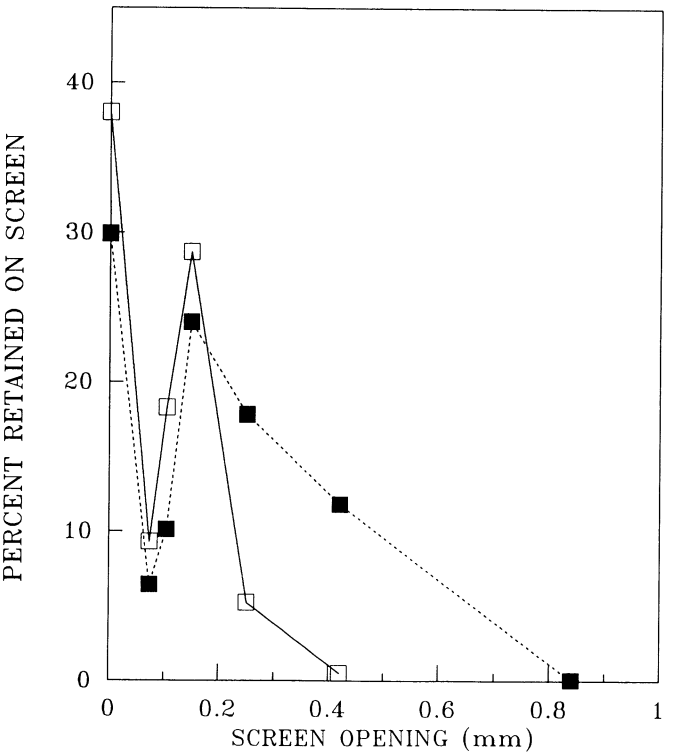

Fig. 2. Particle size distribution of a preparation of alkaline peroxide-treated wheat straw under dry (□) and hydrated (■) conditions. The preparation was fractionated using a series of wire screens as described in the text.

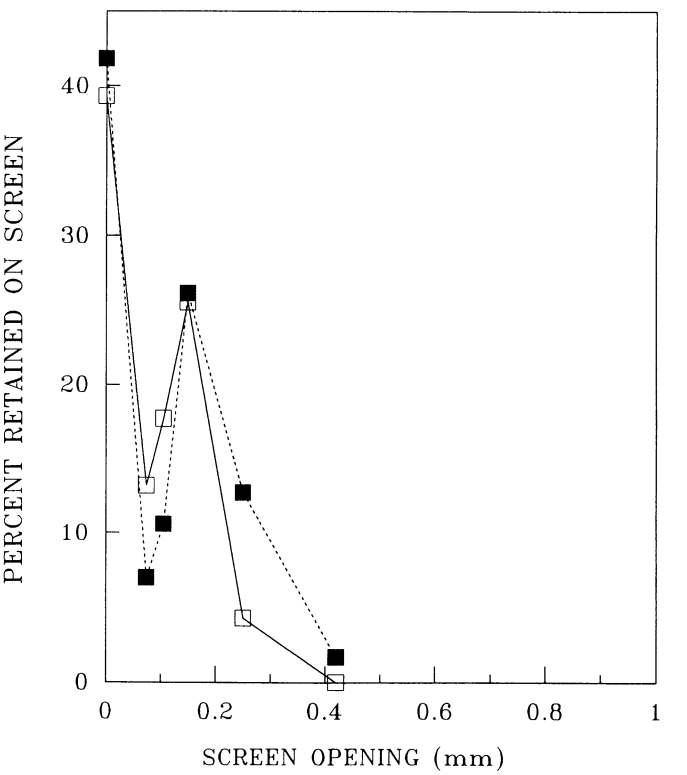

Fig. 3. Particle size distribution of wood pulp cellulose under dry (□) and hydrated (■) conditions. The cellulose was fractionated using a series of wire screens as described in the text.

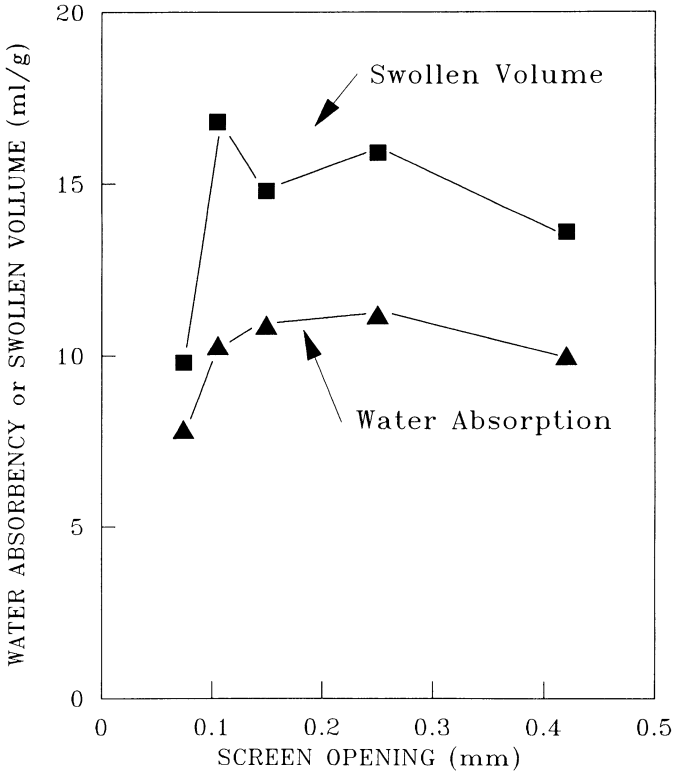

Fig. 4. Effect of particle size on water absorption and swollen volume characteristics of alkaline peroxide-treated wheat straw (AHP-WS). The dry, Wiley-milled sample was fractionated using a series of wire screens as described in Methods. Fractions remaining on the screens with the indicated opening size were assayed for (▲) their ability to absorb water and (■) the volume occupied when hydrated in an excess of water using the procedures described in the text.

presumably because of its higher molecular weight. These data support the notion that AHP-treated plant cell walls have a porous internal structure not found in either untreated cell walls or in highly purified cellulose preparations (Jasberg et al 1989a).

These data suggest that the unique properties of AHP-treated lignocellulose, with regard to its ability to act as a functional replacement for flour in baked foods, can be traced to an open, porous internal structure. Such an open structure would allow more extensive water penetration throughout the lignocellulosic matrix, resulting in more complete hydration of cell wall polysaccharides and a less rigid, more pliant, spongelike structure able to swell, soften, and deform when hydrated. This could account for the lack of gritty textures (Jasberg et al 1989b) and the unusually high degree of functionality (Jasberg et al 1989a) exhibited by AHP-treated lignocellulose in baked foods. It is also consistent with the observation that physical treatments that would destroy an open internal structure (e.g., pressing, ball milling) also greatly reduce the unique functional properties of AHP-treated lignocellulose in foods (Gould et al 1989).

Alkaline hydrogen peroxide treatment selectively removes about half of the lignin and a variable portion of the hemicellulose from plant cell walls (Gould 1984, 1985a). It is possible that the residual, alkaline peroxide-resistant lignin may be functioning as a skeletal framework, maintaining an open structure within the lignocellulose matrix. Purified cellulose preparations such as alpha-cellulose, wood pulp cellulose, and microcrystalline cellulose do not hydrate as extensively as AHP-treated lignocellulose in part because these purified cellulose fractions are extensively delignified, and the original three-dimensional structure of the lignocellulose matrix has collapsed. Untreated (native) cell wall preparations such as brans and hulls also do not hydrate extensively because the amount of lignin present is sufficient to rigidly cross-link the lignocellulose matrix and exclude water from the matrix interior. In both cases, the inability of these materials to become extensively hydrated, swell, and soften results in their functioning as particulate inclusions within a starch-gluten matrix, interrupting and weakening matrix structure

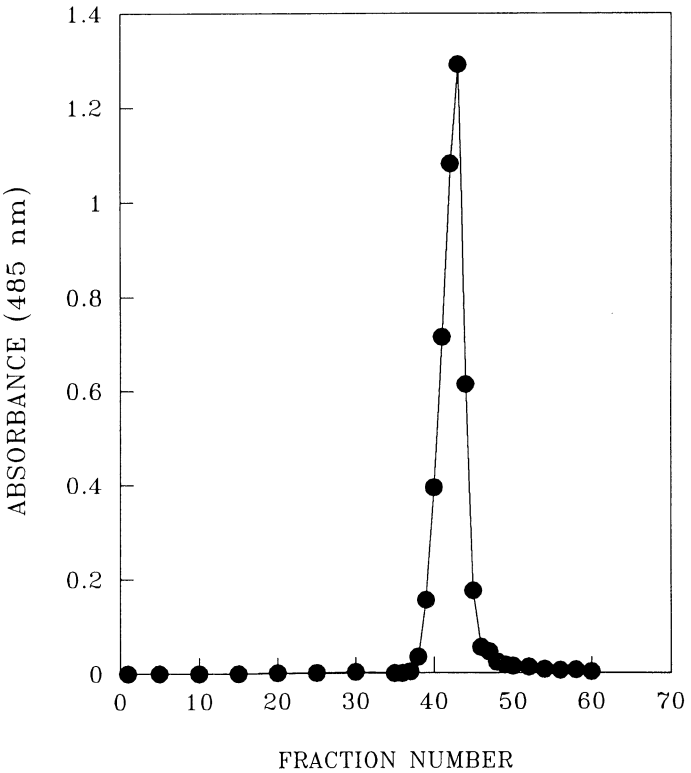

Fig. 5. Elution profile of a mixture of glucose and native B-742-S dextran passed through a 1.0-cm (i.d.) × 46-cm long chromatography column packed with wood pulp cellulose. Total carbohydrate in the eluted fractions (1 ml) was assayed colorimetrically at 485 nm. Details presented in text.

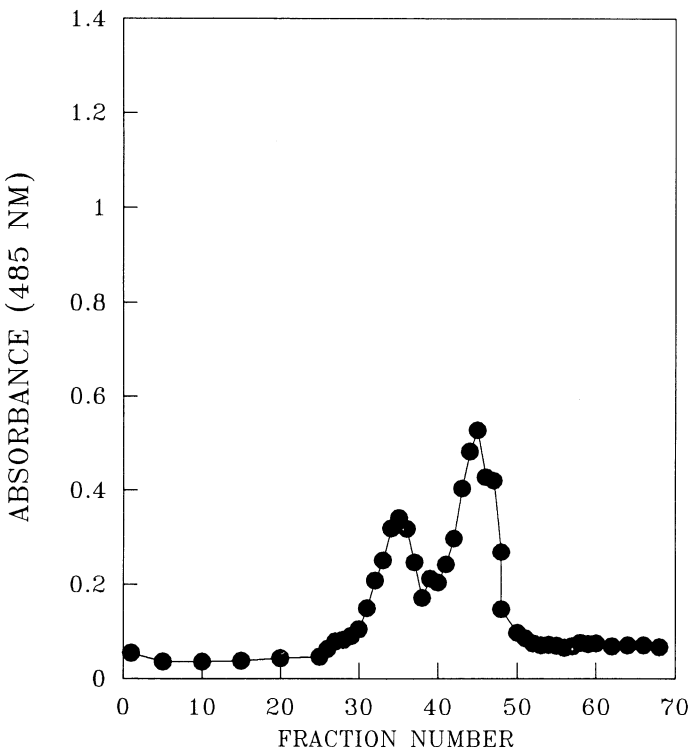

Fig. 6. Elution profile of a mixture of glucose and native B-742-S dextran passed through a 1.0-cm (i.d.) × 46-cm long chromatography column packed with alkaline hydrogen peroxide-treated wheat straw. Total carbohydrate in the eluted fractions (1 ml) was assayed colorimetrically at 485 nm. Details presented in text.

(Dubois 1978, Shogren et al 1981) and introducing gritty textures (Pomeranz et al 1976, Prentice and D'Appolonia 1977). This is in contrast with the partially delignified, extensively hydrated structure of AHP-treated lignocellulose that softens and integrates readily into the starch-gluten matrix with apparently minimal interruption of matrix continuity (Jasberg et al 1989a).

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[Received December 3, 1987. Revision received December 19, 1988. Accepted December 21, 1988.]