Preparation and Effect of Cell-Wall Hydrolysate from Wheat Bran on Mixing Properties of Dough

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Cereal Chem. 71(3):279-282

ABSTRACT

Selective hydrolysis with a macerating enzyme was performed following a steam-pressure treatment to limit degradation of the rigid cell-wall structure. The yield of cell-wall hydrolysate (CWH) was ~25% of the washed bran, with the component xylose comprising ~50%. Gel-filtration on size-exclusion high-performance liquid chromatography of the CWH showed that, as macerating time increased, the molecular weight of CWH decreased. Several main peaks were obtained with molecular mass <5,000. The effect of CWH on rheological behavior of doughs was investigated using a mixograph. Compared to control flour, mixograms of the flour with 1% CWH exhibited shorter dough development time and less mixing tolerance.

Wheat bran contains cell-wall polysaccharides, including cellulose and hemicellulose (which is the major component). The hemicellulose component consists mainly of two pentose sugars: arabinose and xylose (Adams 1955, Schmorak et al 1957). Wheat bran arabinoxylan has a structure characterized by a β-(1,4)-xylan backbone with branching α-L-arabinofuranosyl groups (Brillouet et al 1982, Brillouet and Joselean 1987). Some authors have reported that pentosans influence the rheological behavior of doughs and the texture of bakery products (Medcalf et al 1968, D'Appolonia 1971, Shogren et al 1987).

To isolate hemicellulose (pentosans) from wheat bran, new techniques were developed. A number of investigators have separated wheat bran into various polysaccharide fractions according to their solubilities in different solvents (Schweitzer and Wursch 1979, Ring and Selvendran 1980, Anderson and Clydesdale 1980, Brillouet et al 1982). Previously (Shiiba et al 1992), we reported the development of a new method for large-scale preparation of water-soluble hemicellulose (WSH) from wheat bran. We also investigated the general properties of WSH and separated it into two arabinoxylans (AX-1 and AX-2) by a diethylaminoethyl (DEAE)-Sepharose CL-6B column. AX-1, which passed through that column, was hydrolyzed by endo-1,4-β-D-xylanase, although that enzyme did not work for AX-2, which was adsorbed in the column (Shiiba et al 1993).

This article reports a new method for large-scale preparation of the cell-wall hydrolysate (CWH) from wheat bran. It also reports the results of mixograph studies showing the effect of CWH on rheological behavior of doughs.

MATERIALS AND METHODS

Wheat Bran

Wheat bran obtained from hard spring wheat was provided by Nisshin Flour Milling Co., Ltd. Protein content (N × 5.7) was 14.5%, moisture 14.8%, and ash 4.35%, as determined by approved methods (AACC 1983). Wheat bran was stored at −20°C before use.

Enzyme

Cellulase Onozuka RS was purchased from Yakult Co., Ltd. (Tokyo). This macerating enzyme derived from *Trichoderma viride*. The enzyme contains at least 16,000 units/g of filter-paper-decomposing activity (Tomita et al 1968). The enzyme is usually used for production of protoplasts of plant tissues such as tobacco leaf (Takebe et al 1968).

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Hydrolysis of Carbohydrate

To the carbohydrate samples (10 mg), 5 ml of 2*N* trifluoroacetic acid (TFA) was added. The samples were bubbled for 60 sec with a steady stream of nitrogen. Then they were immediately sealed and placed in an oven at 105°C for 2 hr. The hydrolyzed solution was cooled and centrifuged at 3,000 × *g* for 10 min. Supernatant (2 ml) was evaporated on a rotary vacuum evaporator at 50°C to remove the TFA. The dry matter was dissolved in 0.8 ml of distilled water. Aliquots were analyzed by high-performance liquid chromatography (HPLC). All samples were run in duplicate.

Analysis of Sugar Composition

Aliquots of the hydrolyzed carbohydrate were filtered through a 0.45-μm filter. Filtrates (20 μl) were directly analyzed at 80°C by HPLC using a chromatograph (model 655A-12, Hitachi) equipped with a solvent-delivery system with a model L-5000LC controller, a model 655A-40 automatic sample injector, and refractometer (Shodex RI SE-61, Showa Denko). The column was packed with 10-μm silica (Shodex KS-801P 3 × 200 mm, Showa Denko). Data were recorded and quantified with a Hitachi Chromato Integrator D-2000. Superpurified water was produced (Mill-Q Labo, Millipore) and used as the eluent; the flow rate was 0.7 ml/min. The eluent was degassed online (model 546B, GL-Science, Tokyo). Glucose, xylose, and arabinose concentrations in fractions were estimated from standard curve of the quantified peak area. Pentosan content was determined as the combined concentration of arabinose and xylose.

Determination of Uronic Acids

Uronic acids were determined by the meta-phenylphenol method using glucuronic acid as a standard (Blumenkranz and Asboe-Hansen 1973).

Determination of Phytic Acids

Phytic acid was determined according to the method of Tangendjaja et al (1980). An HPLC system was used as described above. A reverse-phase C-18 column (4 × 300 mm) was obtained from GL-Science. Pure phytic acid was purchased from Wako (Tokyo).

Size-Exclusion HPLC

To investigate molecular weight distribution of the fraction samples, size-exclusion HPLC (SE-HPLC) was performed using the HPLC system described above. The double-connected Ultrahydrogel 250 column (7.8 × 300 mm × 2, Waters) was used for separation of polysaccharides in these experiments. The molecular weight of the polysaccharide was estimated by using a calibration plot of elution time versus the molecular weight of standard pullulans (Shodex pullulan standards P-82, Showa

Denko). The molecular weight markers of pullulan were: P-200 (1.86×10^5); P-100 (1×10^5); P-50 (4.8×10^4); P-20 (2.37×10^4); P-10 (1.22×10^4); and P-5 (0.58×10^4). Superpurified water was used as the eluent; the flow rate was 0.5 ml/min. After being filtered through a 0.45-μm filter, 100 μl of 2% carbohydrate sample solution was applied.

Operation of Mixograph

Mixograms were obtained in an air-conditioned room maintained at 25 ± 1°C using a mixograph (National Mfg. Co, Lincoln, NE) operating at 87 rpm and at spring setting of 9 (Johnson et al 1946). Flour samples (30 g) were mixed at 60% absorption. Fraction samples extracted were added to bread flour at a level of 1.0%, based on flour weight. The flour used was an untreated commercial blend milled by the Nisshin Flour Milling Co., Ltd. Protein content was 13.0%, moisture 14.4%, and ash 0.48%, as determined by approved methods (AACC 1983).

Statistical Analysis

Data were statistically analyzed on a personal computer (PC 9801 VX, NEC Co., Japan) by using the commercially available software program developed by Social Survey Research Information Co., Japan.

RESULT AND DISCUSSION

Preparation of CWH from Wheat Bran

To extract hemicellulose from plant cell-wall material such as wheat bran, alkaline solution is generally used as a solvent. However, the alkaline solution and the resulting salt were very difficult to remove from the products. Using ion-exchange resins and ultrafiltration takes much time. The present study developed a method to extract these hemicellulose materials without using an alkaline solution.

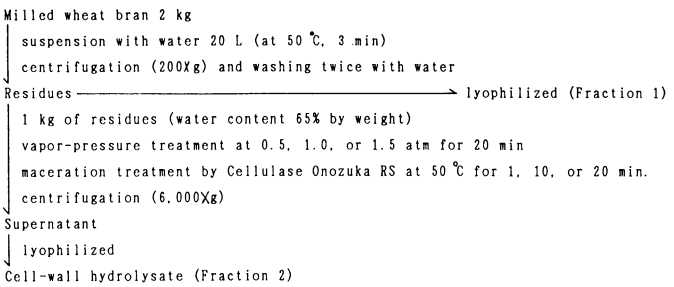

Fig. 1. Fractionation scheme for cell-wall materials from wheat bran.

CWH is prepared in four steps. Endosperm materials are removed from wheat bran with a steam-pressure treatment to limit degradation of the rigid cell-wall structure. Then cell-wall materials are selectively hydrolyzed with a macerating enzyme. Finally, CWH is fractionated into a water-soluble fraction by centrifugation (Fig. 1).

Step 1: Removing starch and protein. To remove soluble protein and starch, 2 kg of wheat bran was suspended in 20 L of warm water at 50°C. The mixture was vigorously stirred in a 45-cm agitator (model Super F, Nisshin Engineering, Tokyo) at 3,000 rpm for 3 min. After agitation, solid matter was separated from the solution by means of a centrifugal filter (model O-20, Tanabe Tekko). The residue was washed twice with water.

Step 2: Steam-pressure treatment. One kilogram of the residues obtained (water content of 65% by weight) were treated in a jar fermentor (model MBU-50, Tokyo Rika) at 1.5 atm (110°C), 2.0 atm (120°C), or 2.5 atm (127°C) for 20 min.

Step 3: Macerating treatment. After steam-pressure treatment, the suspension was stirred with 1 g of cellulase (Onozuka RS) at 50°C, using the same jar fermentor described above, at 600 rpm for 1, 10, or 20 min. Enzymatic reaction was immediately stopped by adding steam to the jar fermentor.

Step 4: Separation of CWH. The suspension was continuously centrifuged at 6,000 × *g*. The supernatant was immediately lyophilized, ground, and sieved by impact mill (described above) to obtain the CWH powder.

Composition of Prepared CWH

CWH was obtained from the washed wheat bran after the steam-pressure and macerating treatments (Table I). However, one treatment alone, either steam-pressure or macerating treatment, did not provide large amounts of CWH and pentosans. These results suggested both treatments were necessary to extract pentosans.

As pressure was increased, the CWH yield increased, but the carbohydrate components in the CWH changed only slightly. However, because of higher extraction ratio of pentosans (xylose and arabinoise) and total carbohydrate, using the 2-atm steam-pressure treatment was adequate to fractionate CWH. As maceration time was increased, the yield of CWH also increased, and the extraction ratio of carbohydrate in the CWH was slightly decreased, depending on the xylose and glucose content. Short maceration time (1–10 min) seemed adequate to fractionate high concentrations of pentosans. Optimal conditions for fractionating high concentrations of pentosans in CWH, in spite of low dry weight yield, were 2 atm of steam-pressure and 1 min of macerating treatment. The sugar composition of CWH showed pentosans to be a major component (~60%) of CWH, of which arabinose was a minor component (<10%). The small extraction rate of

TABLE I
Percentage of Yield (Dry Weight), Sugar Composition, and Pentosan Content (Xylose and Arabinose) of Cell-Wall Hydrolysate (CWH) Obtained by Fractionation[a]

Treatment							
SP[b] (atm)	MT[c] (min)	Yield[d]	Sugar Composition: Xylose	Arabinose	Glucose	Total Carbohydrate	Pentosan Content
1.5	1	17.7 ad	48.8	7.5	20.5	76.8	56.3
1.5	10	21.0 bd	50.1	6.8	19.5	76.4	56.9
1.5	20	26.0 cd	47.7	7.9	20.8	76.4	55.6
2.0	1	20.2 ade	56.4[e]	9.1[e]	21.9[e]	87.4[e]	65.5[e]
2.0	10	25.0 bde	51.6	7.5	20.4	79.5	59.1
2.0	20	27.8 cde	50.1	7.1	19.9	77.1	57.2
2.5	1	23.2 ae	46.5	7.5	20.7	74.7	54.0
2.5	10	27.0 be	51.6	8.3	20.6	80.5	59.9
2.5	20	28.0 ce	46.2	6.4	19.4	72.0	52.6
Control							
2.0	0	5.1	11.3	3.8	34.2	49.3	15.1
0	20	2.7	5.4	2.7	29.3	37.4	8.1

[a] Values are the average of two replicates. All duplicates were within 5% of the mean.
[b] SP steam-pressure treatment.
[c] MT maceration treatment.
[d] Yield of dry weight from the washed wheat bran. Means within the same group exhibiting the same letter are not significantly different ($P = 0.95$).
[e] Significant difference ($P < 0.05$) between the marked value and others.

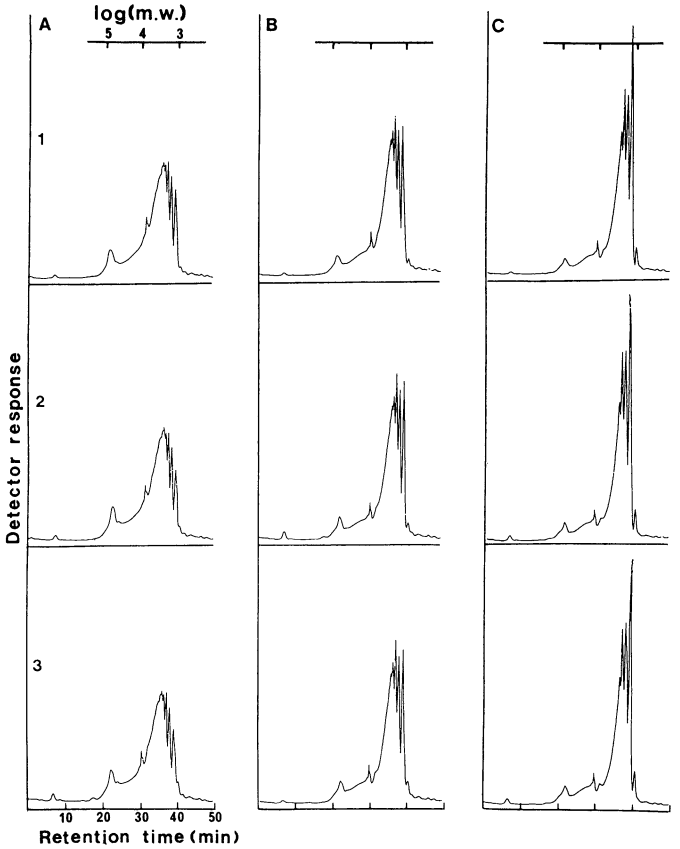

Fig. 2. Elution profiles of cell-wall hydrolysate (CWH) by size-exclusion high-performance liquid chromatography with the double-connected Ultrahydrogel 250 column at a flow rate of 0.5 ml/min using superpurified water as the eluent. The logarithmic molecular weights (upper axis) were estimated from pullulan standards. CWH, made from wheat bran with macerating treatments at 1, 10, and 20 min, respectively, is shown in **A–C** . **1–3** refer to different steam-pressure treatments (1.5, 2.0, and 2.5 atm, respectively).

arabinose might be caused by the specificity of xylanase in the macerating enzyme. Xylanase is specific for the xylose residues that are devoid of branches of L-arabinofuranose residues or 2-O-β-D-xylopyranosyl-L-arabinose units in the arabinoxylan. Although high amounts of glucose (~20%) were also detected, that might be the result of hydrolysis of hemicellulose as well as cellulose. It has been reported that one hemicellulose fraction (AX-1) consisted of glucose in addition to xylose and arabinose (Shiiba et al 1993). No uronic acid or phytic acid was detected in samples of CWH.

The gel-filtration chromatography profiles of the CWH on SE-HPLC are shown in Figure 2A–C for 1, 10, and 20 min of maceration, respectively. The maceration treatment caused changes in molecular weight distribution of CWH. The maceration time of 1 min (Fig. 2A) produced a broad peak, which corresponded to a molecular mass distribution of ~100:1 kDa of pullulan. However, as maceration time increased, the molecular weight distribution of CWH became narrower to give an average molecular weight lower than 5,000.

Mixograms of Flours Containing CWH

As shown in Figure 3, mixograms of the flour with CWH exhibited shorter dough development time and less mixing tolerance than did those of the control flour (without CWH). In particular, there was a pronounced effect for the flour containing the higher molecular weight CWH. These results suggested that high molecular weight pentosans might influence dough rheology.

D'Appolonia previously described the effects of pentosans on farinograph mixing properties (D'Appolonia 1971). However, he noted that the effect of pentosans on the mixing properties may

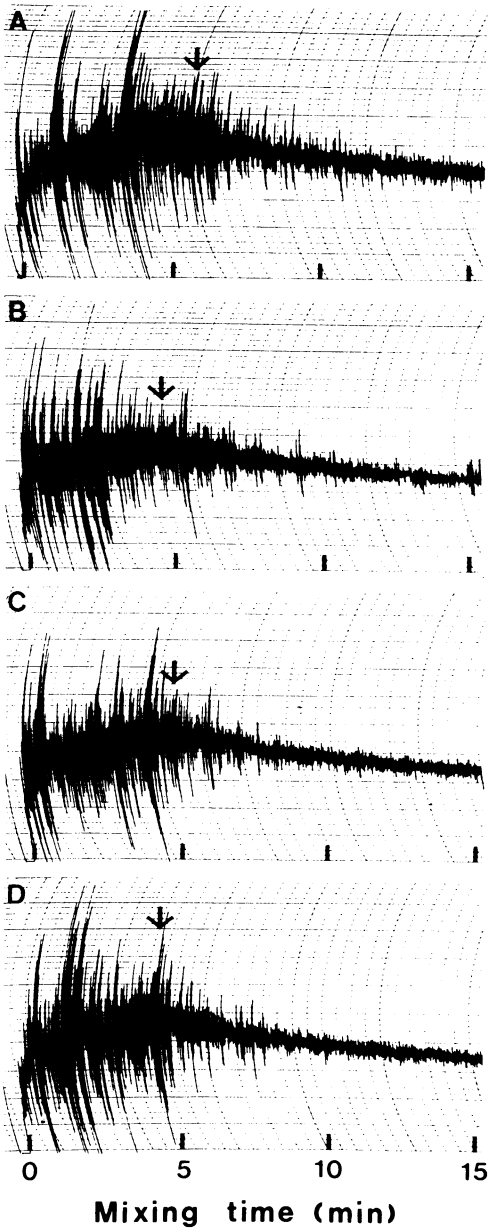

Fig. 3. Mixograms showing effects of cell-wall hydrolysate (CWH) on supplemented flour. **A,** control flour without CWH; **B–D,** flours supplemented with 1% CWH made from wheat bran at 1.0 atm of steam-pressure treatment and macerating treatment for 1, 10, and 20 min, respectively. Arrows indicate peak of mixing development. Dough development times for **A–D** were 5.8, 4.3, 4.7 and 4.6 min, respectively.

differ because of the preparation method. In this study, CWH was responsible for a reduction in development time during mixing, a reduction similar to those found by Pence et al (1950) and Shogren et al (1987).

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[Received August 26, 1993. Accepted January 27, 1994.]