Preparation and Characterization of Water-soluble Hemicellulose (Arabinoxylan) from Wheat Bran

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Water-soluble hemicellulose (WSH) extracted from wheat bran by homogenizing with dilute (0.2M) NaOH solution was purified by ultrafiltration and ion-exchange resins. The yield of the final product (WSH) was 7.6% of the bran. The molecular weight distribution of WSH, which was determined by gel filtration chromatography on size-exclusion HPLC, showed that the WSH consisted of polysaccharides of wide molecular weight distribution with a peak of around 350,000 dalton. The analysis of sugar composition showed that the WSH was a highly substituted arabinoxylan in which the ratio of arabinose to xylose was 0.91. The viscosity of the aqueous WSH solution was exponentially correlative to the WSH concentration. WSH was unstable on the condition of low pH (pH 3 and 5) and high temperature (80-100°C), but it was stable at higher pH (pH 9), even at high temperature (80-100°C). It was also shown that WSH had a unique characteristic, a sustained release function, which had never been demonstrated in other polysaccharides such as starch and cellulose. By this function, the tablet comprising bromphenol blue within a matrix of WSH could gradually release this substance into the solution as the tablet was being dissolved in the aqueous phase. The effect was observed even with a tablet of a mixture containing only 20% WSH with 80% cellulose.

Wheat bran contains cell-wall polysaccharides, hemicellulose and cellulose. In these cell-wall polysaccharides, hemicellulose (arabinoxylan) is the main component, which mainly consists 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 branched α-L-arabinofuranosyl groups and short chains of 2-, 3-, 5-, and 2, 3- linked arabinose residues (BRILLOUET et al., 1982; BRILLOUET and JOSELEAU, 1987). Studies have also been made on the chemical, physical, and nutritive qualities (SOUTHGATE et al., 1976; D’APPOLONIA and MACARTher, 1976; CUMMINGS et al., 1978; NOMANI et al., 1979; BERTRAND et al., 1981; FINCHER and STONE, 1986; MEUSER and SUCKOW, 1986). Some authors reported that pentosans might influence the rheological behavior of doughs and the texture of bakery products (MEDCALF et al., 1968; D’APPOLONIA, 1971; SHOGREN et al., 1987).

In order to fractionate arabinoxylan from wheat bran, new techniques were developed. A number of investigators separated wheat bran into various polysaccharide fractions according to their solubilities in different solvents (SCHWEITZER and WURSCH, 1979; RING and SELVENDRAN, 1980; ANDERSON and CLYDEDALE, 1980; BRILLOUET et al., 1982). Most of these reports were concerned with the small-scale preparation and limited characterization of the arabinoxylan.

In this study, a new method for large-scale preparation of the major arabinoxylan from wheat bran was developed, and the general properties (molecular-weight distribution, viscosity, pH and thermal sensitivity), sugar composition, and sustained release function
were investigated.

Materials and methods

Wheat bran

Wheat bran obtained from hard spring wheat was provided by Nisshin Flour Milling Co., Ltd. The protein content (Kjeldahl method) was 14.5%, moisture 14.8%, and ash 4.35%. The wheat bran was stored at -20°C before use.

Preparation of water-soluble hemicellulose (WSH)

Soluble hemicellulose was prepared as summarized in Fig. 1. Details of the individual steps are as follows.

Step 1. In order to remove soluble protein and starch, wheat bran (2 kg) was suspended in 20 l of warm water at 50°C and the mixture was vigorously stirred by a large-scale agitator (model Super F, Nisshin Engineering) at a circumferential speed of 25 m/sec for 3 min. After completion of the agitation, the solid material was separated from the solution by means of a centrifugal filter (model 0-20, Tanabe Tekko). The residue was washed twice with water. Part of the residue (50 g) obtained was immediately lyophilized, ground and sieved by an impact mill (Retch Ultra Centrifugal Mill with the 0.5 mm filter) to obtain a powder (fraction 1).

Step 2. The residues obtained (water content of about 50% by weight) were suspended in 10 l of 0.2 M NaOH at 80°C, and the mixture was stirred using the same agitator as described above at a circumferential speed of 20 m/sec for 1.5 h. The suspension was continuously centrifuged at 6,000 × g. The supernatant (crude extract of water soluble hemicellulose) was neutralized by adding 2 M HCl. Part of the supernatant (50 ml) was immediately lyophilized, ground and sieved by the impact mill described above to obtain a powder (fraction 2).

Step 3. After adding 10 l of water to the supernatant containing hemicellulose, the solution was dialyzed against water for 3 h by an ultrafiltration system (model RUW-2, Nitto Denko) equipped with a polysulfonic membrane UF-3520 (model P-18, total membrane area of 0.76 m², inner diameter of 11.5 mm, Nitto Denko) having a molecular weight cutoff of 20,000 d and concentrated by the same system.

Milled wheat bran 2 kg

| suspension with water 20 l (at 50°C, 3 min) | centrifugation (200×g) | washing twice with water |

Residues lyophilized

| homogenize with 0.2 M NaOH 10 l (at 80°C, 1.5 h) | neutralization by 2 M HCl | continuous centrifugation (6,000×g) |

Supernatant (crude hemicellulose) lyophilized

| addition of water 10 l | ultrafiltration (at 50°C) through UF3520 membrane |

Permeate lyophilized

| deionization by ion-exchange resins |
| (cation-exchange : IR-120 B, anion-exchange : IRA-93) |
| lyophilized |

Purified Soluble Hemicellulose (WSH)

(Fraction 4)

Fig. 1 The scheme of isolation and purification of water-soluble hemicellulose from wheat bran
to obtain 5 l concentrate. The flow rate of solution and pressure against membrane was 13 l/min and 8 kg/cm², respectively. During dialysis, the flux was maintained at 20 l/h • m², and the temperature of the solution was kept at 50°C by an electronic heater with a thermostat. Part of the concentrated solution (50 ml) was immediately lyophilized, ground and sieved by the impact mill described above to obtain a powder (fraction 3).

Step 4. The concentrated solution was deionized by an ion-exchange system with 500 ml each of cation and anion exchange resins (model IR-120 B and IRA-93, respectively, Organo). The flow rate of the solution was 125 ml/min. The retentate obtained was immediately lyophilized, ground, and sieved by the impact mill described above to obtain the carbohydrate isolates, water-soluble hemicellulose (WSH) (fraction 4).

Hydrolysis of carbohydrate
To the carbohydrate of fraction 1–4 (10 mg), 5 ml of 2N trifluoroacetic acid (TFA) was added and a steady stream of nitrogen was bubbled for 60 sec. The samples were immediately sealed and placed in an oven at 105°C for 2 h. The hydrolyzed solution were cooled and centrifuged at 3 000 × g for 10 min. Two ml of supernatant was evaporated at 50°C to remove TFA on a rotary vacuum evaporator, and the dry material 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 passed through a filter of 0.45 µm pore size and the filtrates (20 µl) were directly analyzed at 80°C by HPLC using a chromatograph (Hitachi model 655 A–12) equipped with a solvent-delivery system controlled by a model L-5000 LC controller, a model 655 A–40 automatic sample injector, and a refractometer (Shodex RI SE-61, Showa Denko). The column packed with silica of 10 mm particle size (Shodex KS-801 P 3 × 200 mm, Showa Denko) was used. Data were recorded and quantified with a Hitachi Chromato Integrator D-2000. Super-purified water produced by Milli-Q Labo (Millipore) was used as the eluent at a flow rate of 0.7 ml/min over 20 min. The eluent was degassed on line by a degasser model 546 B (Gaschro Kogyo). Glucose, xylose, and arabinose concentrations in fractions 1~4 were estimated from a standard curve of the quantified peak area. The arabinoxylan content was determined as the combined weight 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). HPLC system used was described above. A reverse phase C-18 column (4 × 300 mm) was obtained from Gaschro Kogyo (Tokyo, Japan). Pure phytic acid was purchased from Wako (Tokyo, Japan).

Size-exclusion HPLC
In order to investigate the molecular-weight distribution of the water-soluble hemicellulose (WSH), size-exclusion HPLC was carried out using the same HPLC system as described above. The Waters Ultrahydrogel 1 000 column (7.8 × 300 mm) used in these experiments has a claimed separation range of 10~1 000 kDa for polysaccharides. The molecular weight of the polysaccharide was estimated 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 used were: P–800 (8.53 × 10⁵), P–400 (3.8 × 10⁵), P–200 (1.86 × 10⁵), P–100 (1 × 10⁵), P–50 (4.8 × 10⁴), P–20 (2.37 × 10⁴), P–10 (1.22 × 10⁴), and P–5 (0.58 × 10⁴). Super-purified water was used as the eluent at a flow rate of 0.5 ml/min. After being filtered (through 0.45 µm of pore size), 100 µl of 3% carbohydrate sample solution was applied. The effluent was collected in 0.5 ml fractions, and the total carbohydrates in the fractions were determined by measuring.
the absorbance at 480 nm according to the phenol-sulfuric acid method described by Dubois et al., (1956).

**Determination of viscosity**

The viscosity of the WSH solution at various concentrations (1 to 5%) was measured with an Ostwald viscosimeter (Iwaki, Tokyo) in a water bath the temperature of which was controlled at 25, 50, and 70°C.

**Determination of pH and thermal sensitivities of WSH**

The pH and thermal sensitivity of WSH was determined as the relative ratio of total carbohydrates to reducing sugars. The temperature (20°C to 100°C) and pH of the buffer solution were changed during incubation with WSH (10 mg/ml). The buffers used were 50 mM citrate buffer (pH 3 and 5) and 50 mM tris-HCl buffer (pH 7 and 9). Total carbohydrates were measured according to the method of Dubois et al. (1956) using D-xylose as a standard. Reducing sugars were measured according to the method of Somogy (1952) and Nelson (1944) using D-xylose as a standard.

**Analysis of the sustained release function of WSH**

A mixture of 5 mg of bromphenol blue and 240 mg of WSH was tabletted using a 8 mm punch-and-die at a pressure of 100 kg/cm. The tablets were tested for elution by the paddle method (rotation of 100 rpm) using deionized water (900 ml) as the elution test solution (USP, 1977). The sustained release function was determined by measuring the absorbance at 254 nm in the eluate and indicated as the rate (%) of elution.

In order to compare with the sustained release function for other sorts of polysaccharides, beta-starch (PC-1000, Junsei Sangyo), crystalline cellulose (Avicel 301, Asahi Kasei), and the mixture of WSH and crystalline cellulose (WSH content: 16.7, 20, 30, 50, and 66.7 % by weight) were used.

**Result and Discussion**

**Analysis of fractions in preparation step of water-soluble hemicellulose (WSH)**

As shown in Table 1, glucose, xylose and arabinose were the major monosaccharides in the bran. The pentose composition (the A/X ratio of xylose to arabinose is 0.58) of hemicellulose from native bran was in good agreement with previously reported data: Brilhouët and Mercier (1981) indicated that A/X was 0.60, Fraser and Holmes (1959) indicated that A/X was 0.51.

The yield of fraction 1 was 56.4%, as shown in Table 2. Analytical data for fraction 1 are shown in Table 1 and 2. These results suggested that the pentose content was changed very

<p>| Table 1 Composition of polysaccharidic fractions obtained by the fractionation procedure |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Sugar composition*&lt;sup&gt;a&lt;/sup&gt;&lt;br&gt;Crude*&lt;sup&gt;b&lt;/sup&gt;protein (%)&lt;br&gt; (%)</th>
<th>Xylose</th>
<th>Arabinose</th>
<th>Glucose</th>
<th>Uronic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native bran molar ratio</td>
<td>19.9</td>
<td>11.6</td>
<td>16.8</td>
<td>5.9</td>
</tr>
<tr>
<td>Fraction 1 molar ratio</td>
<td>30.8</td>
<td>20.0</td>
<td>6.0</td>
<td>7.8</td>
</tr>
<tr>
<td>Fraction 2 molar ratio</td>
<td>26.2</td>
<td>23.8</td>
<td>5.8</td>
<td>8.1</td>
</tr>
<tr>
<td>Fraction 3 molar ratio</td>
<td>41.9</td>
<td>39.2</td>
<td>5.7</td>
<td>8.3</td>
</tr>
<tr>
<td>Fraction 4 molar ratio</td>
<td>44.9</td>
<td>41.5</td>
<td>6.8</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.93</td>
<td>0.14</td>
<td>0.09</td>
</tr>
</tbody>
</table>

* Values are the average of two replications.

* All duplicates were within ±5% of the mean.
Table 2  Yield and recovery of dry weight, pentose content (xylose + arabinose), and crude protein of fractions from preparation step

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Dry weight(^{a,b}) Yield (%)</th>
<th>Pentose content(^{a,b}) (%)</th>
<th>Recovery</th>
<th>Crude protein(^{a,b}) (%)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native bran</td>
<td>100</td>
<td>31.5</td>
<td>100</td>
<td>13.5</td>
<td>100</td>
</tr>
<tr>
<td>Fraction 1</td>
<td>56</td>
<td>28.7</td>
<td>91.1</td>
<td>4.63</td>
<td>34.3</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>22</td>
<td>11.1</td>
<td>35.2</td>
<td>2.18</td>
<td>16.1</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>9.8</td>
<td>7.9</td>
<td>25.1</td>
<td>1.05</td>
<td>7.8</td>
</tr>
<tr>
<td>Fraction 4</td>
<td>7.6</td>
<td>6.6</td>
<td>20.9</td>
<td>0.55</td>
<td>4.1</td>
</tr>
</tbody>
</table>

\(^{a}\) Values are the average of two replications.

\(^{b}\) All duplicates were within ±5% of the mean.

little by washing (recovery 91.1%). The decrease in glucose was due to destarching from the native wheat bran, also the decrease in protein content (recovery 34.3% from native bran) indicated the removal of water-soluble protein by washing with water. In order to remove starch from the wheat bran, enzymatic hydrolysis of the starch is generally used (BRILLIOUET and MERCIER 1981). In this study, the treatment was omitted because there was little difference between the qualities of the final WSH. The pentose content (50.8%) in fraction 1 was higher than that in the wheat bran (31.5%), but the ratio of arabinose to xylose did not change.

From the washed wheat bran, crude hemicellulose was extracted at high temperature (80°C) by homogenizing with dilute (0.2 M) NaOH solution, and the yield of extract (fraction 2) was 22.1% of the original bran. This low extraction rate of crude hemicelulose was due to the difficulty of extraction of water-insoluble hemicellulose by this method, which means that insoluble hemicellulose remained in the extraction residues. Low recovery (35.2% from the original bran) of pentose reflected this phenomenon. Actually, the residues contained high amounts of insoluble hemicellulose (47.3%) which consisted of xylose (35.2%) and arabinose (12.1%). However, it seems that 0.2 M NaOH is the optimum concentration for extracting water-soluble hemicellulose because, as shown in Fig. 2, the maximum amount of the polysaccharides was extracted from the wheat bran when 0.2 M NaOH was used for extraction. Also the use of minimum alkaline concentration for extraction could make the following deionization easy.

In this study, the crude hemicellulose solution was further purified by ultrafiltration and ion-exchange resins. The yield of fractions 3 and 4 from the native bran was 9.8 and 7.6%, and the pentose content after two purification steps was 81.1 and 86.4%, respectively. These results showed that ultrafiltration was useful for the purification to obtain highly purified hemicellulose (arabinoxylan). However, the low yield showed difficulties in retaining low molecular weight arabinoxylan (<20 000) in the membrane. In addition, the significant decrease in the uronic acid and protein contents of fraction 4 might be due to adsorption of these substances on the ion-exchange resins.

The final product (WSH 152 gram) contained a high amount (86.4%) pentose (44.9% xylose and 41.5% arabinose), a low amount of glucose (6.8%), uronic acid (glucuronic acid) (5.2%), crude protein (7.3%), and ash (0.27%). Though 5.25% of phytic acid was determined in the bran, no phytic acid from WSH was detected. The total (105.7%) of these contents was a little more than 100%, it might be due to integrate overestimation.

The higher ratio of arabinose to xylose (A/X = 0.93) in the final product suggested that arabinoxylan with higher branched arabinose was mainly extracted. BRILLIOUET et al. (1982) reported that a highly branched arabinoxylan
Fig. 2 Changes in total carbohydrates extracted from the wheat bran by various concentrations of NaOH

The washed wheat bran (Fraction 1) was suspended in various concentrations of NaOH at 80°C, and the mixture was homogenized for 1.5 h. After the suspension was centrifuged (6000 × g), the total carbohydrates in the resulting supernatant were determined by the phenol-sulfuric acid method (Dubois et al. 1956).

was precipitated in range of 60–90% ethanol concentration from the crude hemicellulose solution. Ethanol was generally used for the fractionation of hemicellulose (Mares and Stone, 1973; Matheson and Saini, 1977; Saini and Henry, 1989). However, the use of ethanol for the fractionation of hemicellulose was not satisfactory to obtain pure arabinoxylan because of coaggregation with polysaccharides and other materials such as proteinaceous substances or minerals.

Though Brilhouet et al. (1981) reported that hemicellulose B which was purified from wheat bran using the ethanol fractionation method, was composed of 80.1% pentose (43% xylose and 37.1% arabinose), 9.4% glucose, and 4.3% uronic acid, our results for the sugar composition of fraction 4 (final product) were similar to these results. However, further work, such as structural studies, is required to ascertain differences between hemicellulose B and fraction 4.

Molecular weight distribution of polysaccharides of WSH

The gel filtration chromatography profile of WSH on size-exclusion HPLC is shown in

Fig. 3 Elution profile of water-soluble hemicellulose by size-exclusion HPLC with an Ultrahydrogel 1000 column at a flow rate of 0.5 ml/min using super-purified water as the eluent

The effluent was collected in 0.5 ml fractions, and the carbohydrates in the fractions were determined by measuring the absorbance at 480 nm according to the phenol-sulfuric acid method. Vo and Vt represent the void volume and total bed volume for this column, respectively. The axis of molecular weight (upper line) was estimated by the relationship between the logarithm of the average molecular weight of a pullulan standard (Shodex Pullulan Standards P–82) and the retention time.
Fig. 3. The polysaccharide was eluted in a single, symmetric peak between the void volume (Vo) and total bed volume (Vt). The molecular weight distribution of the polysaccharide was estimated using a calibration plot of elution time versus the molecular weight of standard pullulans. The results showed that WSH consisted of polysaccharides having a wide range of molecular weight distribution (1000–200,000). The molecular weight of the peak was about 350,000 and average molecular weight of polysaccharides was about 300,000, which showed a significant difference from 126,000 of wheat flour hemicellulose (Cole 1969).

**Viscosity of WSH solution**

Fig. 4 shows the effect of temperature and concentration on viscosity of WSH solution. As the temperature increased, the viscosity decreased. The viscosity depended exponentially on the concentration. The relationship between viscosity and concentration of the WSH solution at 25°C was defined by the following expression.

\[ Y = 6.10 X^2 - 7.02 X + 5.81 \]

\( Y \) is the viscosity (c.p.) of the solution at 25°C, which is measured by an Ostwald viscosimeter. \( X \) is the concentration (%) of WSH solution.

**pH and thermal sensitivities of WSH**

As shown in Fig. 5, the WSH in the tris-HCl buffer solution (pH 7 and 9) had high stability at any temperature. However in pH 3 and pH 5 buffer solutions, about half of the amount of arabinosyl xylopyranosyl decomposed above 80°C. It suggested that WSH was likely to be hydrolyzed at low pH rather than at high pH.

**Sustained release function of WSH**

As shown in Fig. 6, bromphenol blue tabletted with WSH was gradually eluted into the elution test solution, and this effect (sustained release function) was observed even after 6 h. The other polysaccharides such as beta-starch and crystalline cellulose exhibited no effect at all, because both polysaccharides were completely eluted within 5 min. However, the mixture of WSH and crystalline cellulose, even with 20% contents WSH in mixture, showed sustained release function. These results suggested that soluble hemicel-
Fig. 6 Comparison of the sustained release function between water-soluble hemicellulose (WSH) and other kinds of polysaccharide (beta-starch and crystalline cellulose)

After the mixture of bromphenol blue and polysaccharides was tabletted, the tablets were tested for elution by the paddle method. The sustained release function was determined by measuring the absorbance (254 nm) of bromphenol blue in the elution test solution and calculating the rate (%) of elution. No.1 and 2: 100% beta-starch and 100% crystalline cellulose, respectively, No.3 ~ 7: a mixture of crystalline cellulose and WSH: WSH content 16.7% (3), 20% (4), 30% (5), 50% (6), and 66.7% (7), and 8: 100% of WSH.

lulose from wheat bran consisted of unique matrix structures and it would exhibit the sustained release function.

This function of WSH could be applied in the field of food science as well as agriculture, for example a mixture of WSH and tasty chemical compounds could help to retain the taste over a long period.

References
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