(1→3), (1→4)-β-glucan and Bound Phenolics in Rolled Oats

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Received February 27, 2008; Accepted June 3, 2008

Rolled oats, a processed oat product, contain about 4 g of (1→3),(1→4)-β-glucan (β-glucan) per 100 g. It also contains 27.0 mg of ferulic acid and 3.5 mg of p-coumaric acid. Moreover, the presence of β-glucan, ferulic acid, and p-coumaric acid was confirmed in commercial food products with rolled oats in their lists of ingredients. The radical scavenging activity of a water-soluble polysaccharide fraction (WS) extracted from rolled oats was examined and components involved in this activity were evaluated. This WS exhibited radical scavenging activity and a strong correlation (r=0.9982) was observed between this activity in terms of the ferulic acid equivalent and WS sample concentration. Concerning the neutral sugar composition of the WS, glucose accounted for about 49 mol%, followed by xylose (about 28 mol%) and arabinose (about 15 mol%). In addition, ferulic acid was contained in a macromolecular fraction collected by gel-filtration chromatography of lichenase-treated WS. Therefore, arabinoxylan containing ferulic acid may be a component leading to the radical scavenging activity of the WS.

Keywords: rolled oats, (1→3),(1→4)-β-glucan, bound phenolics, ferulic acid, arabinoxylan, radical scavenging activity

Introduction

Oats, with their high vitamin and mineral contents, are a nutritionally excellent material and an important source of dietary fiber (Johansson et al., 2000; Virkki et al., 2005). Particularly, it has been reported that oats contain β-glucan (Skendi et al., 2003; Weightman et al., 2004) and that β-glucan is effective for improving blood glucose and cholesterol levels (Anderson et al., 1994; Tappy et al., 1996; Behall et al., 1997; Wood et al., 2000; Hallfrisch et al., 2003; Brennan and Cleary, 2005).

Rolled oats are a processed product prepared by steaming for several hours and rolling with further steaming. For use in food, oats are subjected to steaming processes to inactivate enzymes and thereby prevent rancidity during storage of the final oats products (Bryngelsson et al., 2002). Also, rolled oats are thicker, less brittle, and closer in appearance to original oats than oatmeal. Rolled oats are often used as a primary ingredient of cereal. Some rolled oat products are also pre-cooked to produce products with a shorter cooking time. However, reports have mainly focused on whole oat flour and there are few studies concerning rolled oats or processed foods made primarily from them.

Grains are known to contain phenolic acids such as ferulic and p-coumaric acids (Lempereur et al., 1997; Andreasen et al., 2000), which have functions such as antioxidative activity (Sahidi et al., 1992; Ohta et al., 1994; Castellucio et al., 1995; Ohta et al., 1997; Andreasen et al., 2001; Liyana-Pathirana and Shahidi, 2006). Therefore, phenolic acids are considered to be important components of rolled oats. Generally, phenolic acids are bound to polysaccharides by ester bonds in grains (Fry, 1986; Ishii, 1997) and are known as components involved in cell extension and control (Fry, 1986; Kamisaka et al., 1990). Therefore, their detailed structures and functions are considered to require evaluation along with those of polysaccharides.

In this study, therefore, the β-glucan and phenolic acid contents of rolled oats and commercial food products with rolled oats in their lists of ingredient were examined. Moreover, as the first step toward the clarification of the functions of rolled oats, their water-soluble polysaccharide fraction (WS) analyzed. As a result, we clarified that water-soluble polysaccharides in rolled oats exhibit an antioxidative activity and that polysaccharides containing ferulic acid are a

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source of this activity.

Materials and Methods

Samples  The samples were rolled oats prepared from oats harvested in 2005 and 2006 (time of production: 2006 and 2007, respectively) and two different commercial food products (Products F and D) with rolled oats in their lists of ingredients were purchased from a local market.

Rolled oats and these two different commercial food products were milled and about 50 g of each was placed with 100 ml of 80% ethanol in a mixer and homogenized. The homogenates were divided into soluble and insoluble fractions by aspiration filtration. The insoluble fractions were returned to the mixer and the same homogenization-filtration process was repeated twice. Then the residues were washed with acetone and air-dried, and alcohol-insoluble solid fractions (AIS) were obtained. The amount of AIS obtained from rolled oats was 45.7 g and that from the commercial products was 35.0 g.

Analysis of β-glucan and bound phenolics  β-Glucan was assayed in the AIS of rolled oats and the selected commercial food products using a β-glucan assay kit (Biocion, Japan).

Bound phenolics were assayed in the AIS using a reported procedure (Nara et al., 2006). Each AIS (500 mg) was suspended with 1 M NaOH (20 ml) and shaken overnight at room temperature. The supernatant was obtained by centrifugation (3,000 rpm × 15 min), made acidic with hydrochloric acid, and treated with ethyl acetate for extraction. The ethyl acetate layer was recovered, the extraction procedure was performed two more times, and all ethyl acetate layers were pooled. The pooled ethyl acetate layer was evaporated in a vacuum to dryness, redissolved with ethanol, and analyzed by high performance liquid chromatography (HPLC) under the following conditions: instrument, Agilent1100; column, Inertsil ODS-3 (4.6 × 250 mm, GL Science); column temperature, 40°C; mobile phases, (A) 25 mM acetate buffer (pH 3.3) and (B) acetonitrile, 10%(B) → 30%(B) (30 min, linear concentration gradient); measurement wavelength, 325 nm; flow rate, 1.0 ml/min.

Analysis of WS and its neutral sugar composition  The AIS of rolled oats was placed in a centrifuge tube with a stopper, suspended with 50 ml of 20 mM sodium acetate buffer (pH 5.0), 50 units/g AIS of glucoamylase (Rhizopus sp.) and 20 units/g AIS of pullulanase (Klebsiella pneumoniae) were added, and the mixture was incubated at 40°C for 24 hours. After the reaction, the mixture was divided into the supernatant and sediment by centrifugation (3,000 rpm × 15 min). The supernatant was recovered and the same enzyme treatment and centrifugation were performed with the sediment. The sediment was sufficiently washed with distilled water and all supernatants were pooled. The collective supernatant was sufficiently dialyzed against distilled water and the retentate was freeze-dried and used as the final WS.

The WS was hydrolyzed by 2 M trifluoroacetic acid (100°C, 3 hours) and the degradation products were evaporated in a vacuum to dryness. The neutral sugar composition of the hydrolysate was analyzed by high-performance anion-exchange chromatography (HPAEC). This HPAEC was performed with a Dionex DX-500 system (Dionex, Sunnyvale, CA, USA) with a pulsed amperometric detector. The separation column was a CarboPac PA1 (4 × 250 mm), the guard column was a CarboPac PA1 GUARD, and flow rate was 1 ml/min.

Measurement of radical scavenging activity  The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was measured by a reported technique (Nara et al., 2006). To a microplate, 50 µl of a sample diluted with 50% ethanol solution and 150 µl of 0.1 mM DPPH/50% ethanol solution were added; the microplate was shaken at room temperature for 30 min, and the absorbance at 540 nm was measured using a microplate reader (Biorad Model 680). The absorbance was also measured in a mixture of 150 µl of 50% ethanol solution and 50 µl of a sample solution (blank) and a mixture of 50 µl of 50% ethanol solution and 150 µl of 0.1 mM DPPH/50% ethanol solution (control). The radical scavenging activity was expressed as the ferulic acid equivalent.

Enzyme treatment of WS  The WS was treated with xyylanase (Trichoderma viride) and lichenase contained in the (1 → 3), (1 → 4)-β-glucan assay kit.

To the WS (1 mg/1 ml 50 mM acetate buffer, pH 5.5), 2 units of each enzyme were added, the mixture was allowed to react at 40°C for 5 hours, and the reaction was stopped by heating the reaction mixture in a boiling water bath for 10 minutes. After cooling, the reaction mixture was analyzed by Bio-Gel P-2 column chromatography (1.5 × 47 cm) equilibrated with distilled water in advance. Elution was performed with distilled water and the eluate was collected as 3-ml fractions. These fractions were sampled and the total sugar content was determined by the phenol-sulfuric acid method (Dubois et al., 1956).

Results and Discussion

β-glucan and bound phenolics contents of rolled oats  Figure 1 shows the β-glucan contents of rolled oats produced at different times. No significant difference was noted according to the year or day of production, and the mean β-glucan content of the samples was about 4.0 g/100 g of rolled oats. Skendi et al. (2003) reported that oats contained 3-7% β-glucan, values not widely different compared with its content in rolled oats in this study. The β-glucan content
was about 4.0% in oats both before and after steaming and rolling (data not shown), suggesting no marked change in the β-glucan content due to the steaming or rolling process.

Next, the contents of cell wall-bound phenolics in rolled oats were analyzed. Figure 2 shows chromatograms of cell wall-bound phenolics in the AIS of rolled oats by HPLC. The primary phenolic acid contained in the AIS of rolled oats was ferulic acid, the content of which was 27.0 mg/100 g of rolled oats. The p-coumaric acid content was 3.5 mg/100 g. There was no significant difference in the bound phenolic content of oats both before and after steaming and rolling, as well as for the β-glucan content (data not shown). In grains such as barley and wheat, most phenolic acids exist in the bound form (Liyana-Pathirana and Shahidi, 2006; Ishii,
Naczk and Shahidi (1989) recognized the importance of bound phenolics in the total phenolic content. Furthermore, the bound phenolic fraction demonstrated a significantly higher antioxidant capacity than free phenolics in an in vitro antioxidant assay (Liyana-Pathirana and Shahidi, 2006). In rolled oats, the amount of free phenolics may be very low. However, Bryngelsson et al. (1986) reported that free phenolics increased in hydrothermal processes (steaming, autoclaving, and drum drying). Therefore, further study is needed to clarify the changes in free and bound phenolics during processing.

Ferulic acid exhibits an antioxidative activity (Sahidi et al., 1992; Ohta et al., 1994; Castelluccio et al., 1995; Ohta et al., 1997; Andreasen et al., 2001; Liyana-Pathirana and Shahidi, 2006) and has recently been reported to show depressor, anti-tumor, and anti-Alzheimer’s activities (Mori et al., 1999; Yan et al., 2001; Suzuki et al., 2002; Mamiya et al., 2004). Therefore, rolled oats may also exhibit these effects.

Also, chromatograms obtained by HPLC showed some peaks other than those of ferulic and p-coumaric acids, but grains have been reported to contain dimers of ferulic acid (Andreasen et al., 2000; Bunzel et al., 2006). Therefore, the peaks other than those of ferulic and p-coumaric acids may have been those of such dimers (e.g., diferulic acids). Because dimers are also expected to have functions such as antioxidative activity, further evaluation of their functions is necessary.

Next, we evaluated the β-glucan and bound phenolic contents of two different commercial food products (Products F and D) with rolled oats in their list of ingredients, purchased from a local market (Fig. 3). Products F and D contained β-glucan at 1.5 and 2.2 g/100 g, respectively. Bound phenolics were also detected, and the ferulic acid contents were 13.7 and 20.7 mg, and p-coumaric acid contents were 1.6 and 5.1 mg, respectively. It seems that the rolled oat contents and processing methods are respectively different in Products F and D. Because there is insufficient information on the changes of β-glucan and bound phenolics due to processing methods, detailed evaluation is still necessary. However, β-glucan and bound phenolics were contained in Products F and D as well as in rolled oats and these are also considered to have various functions.

Separation of WS and its DPPH radical scavenging activity There have been a number of studies on the antioxidative activity of ferulic acid. Because phenolic acids such as ferulic acid are bound to cell wall polysaccharides by ester bonds in grains including oats, evaluation of the antioxidative activities of phenol-containing polysaccharides is considered to be necessary in grains. We, therefore, separated water-soluble polysaccharides from rolled oats and evaluated their antioxidative activities using the DPPH radical scavenging activity as an index.

Starch was removed from the AIS of rolled oats by enzyme treatment and the low-molecular-weight fractions derived from starch such as glucose were eliminated by dialyzing polysaccharides and starch degradation products solubilized in this process against water. The dialysate was regarded as WS, the amount of which was 38.4 mg/100 mg of AIS.

Figure 4 shows the DPPH radical scavenging activity...
of the separated WS. A strong correlation of $r=0.9982$ was observed between the radical scavenging activity in terms of the ferulic acid equivalent and WS concentration. Therefore, the WS of rolled oats is considered to contain components with radical scavenging activities. Analysis of the neutral sugar composition of the WS (Table 1) showed that glucose accounted for about 49 mol%, followed by xylose (about 28 mol%) and arabinose (about 15 mol%). Therefore, the radical scavenging activity of the WS is contained in these polysaccharides.

**Enzyme treatment of WS** The structures of polysaccharides contained in the WS of rolled oats with radical scavenging activities were evaluated in greater detail by enzyme treatment. The WS was treated with lichenase and xylanase and after inactivation of the enzymes, the enzyme degradation products were separated by gel filtration chromatography using a Bio-Gel P-2 column. Figure 5 shows changes in the elution pattern after enzyme treatment compared with that before enzyme treatment. In the WS without enzyme treatment [Fig. 5(A)], elution was observed only near the

![Fig. 5. Fractionation of enzyme degradation products of the water-soluble polysaccharide fraction of rolled oats.](image)

(A): No enzyme treatment  (B) Xylanase treatment  
(C): Lichenase treatment  (D) Xylanase + lichenase treatment  
AX: Collected macromolecular fraction

| Table 1. Neutral sugar composition in the water-soluble polysaccharide fraction (WS) of rolled oats. |
|---------------------------------------------------------------|---------------------------------------------------------------|
| Fraction | Neutral sugar composition (mol %) |                           |
|          | Arabinose | Galactose | Glucose | Xylose | Mannose  |
| WS       | 15.2      | 5.3       | 49.4    | 27.6   | 2.5      |
void volume of the column. In the WS after treatment with xylanase [Fig. 5(B)] and lichenase [Fig. 5(C)], mono- and oligosaccharides were noted in addition to the peak near the void volume of the column. When the WS was treated simultaneously with xylanase and lichenase [Fig. 5(D)], the peak near the void volume was inconspicuous and most of the eluted sugars were mono- and oligosaccharides. From these results and the neutral sugar composition of the WS, the WS is considered to contain xylan groups (e.g., arabinoxylan), and glucan groups (e.g., \((1 \to 3), (1 \to 4)-\beta\)-glucan), polysaccharides with very low contents of other polysaccharides. From the neutral sugar composition, glucan-group polysaccharides such as \((1 \to 3), (1 \to 4)-\beta\)-glucan containing glucose (about 49 mol%) and xylan-group polysaccharides such as arabinoxylan containing arabinose (about 15 mol%) and xylose (about 28 mol%) may account for similar percentages.

Feruloyl oligo- and polysaccharides are known to exhibit antioxidant activity in vitro (Ohta et al., 1994; Ohta et al., 1997). Because ferulic acid, a major bound phenolic acid, is known to exist as ester-linked mainly to arabinoxylan, arabinoxylan containing phenolic acid is considered to be the antioxidative component of the WS obtained in this study. Phenolic acid in the WS was analyzed using a lichenase-treated high-molecular-weight fraction of WS [Fig. 5(C), AX]. Figure 6 shows chromatograms of bound phenolics in AX. A peak with a retention time in agreement with that of ferulic acid standard was observed in AX.

From the results in Figure 5, there are xylan-group polysaccharides in AX, a lichenase-treated high-molecular-weight fraction. Therefore, xylan-group polysaccharides containing ferulic acid may be the components of the WS involved in its DPPH radical scavenging activity.

The DPPH radical scavenging activity is the sum of the activities of various components, and arabinoxylan containing ferulic acid is considered to be a component of the WS of rolled oats involved in its radical scavenging activity. However, the DPPH radical scavenging activity estimated from the ferulic acid content of the WS was only about 1/8 of the total activity. There are reports that the antioxidative activity was higher in ferulic acid bound to oligo- or polysaccharides than in free ferulic acid (Ohta et al., 1994; Ohta et al., 1997; Rao and Muralikrishna, 2006). Therefore, the total radical scavenging activity is considered to have increased in this study because polysaccharides bound to ferulic acid were contained in the WS assayed for radical scavenging activity.

In this study, the WS was evaluated and the amount of ferulic acid in the WS was 1.3 mg per 100 g of rolled oats. Because this accounted for only about 5% of the total ferulic acid content, a high percentage of ferulic acid is considered

![Fig. 6. Chromatograms of bound phenolics in the macromolecular fraction (AX) obtained by lichenase treatment of the water-soluble polysaccharide fraction. (a) Ferulic acid.](image-url)
to have been present in the WS. Therefore, further evaluation of this WS is necessary.

References


