Carbohydrate Polymers 81 (2010) 784–789

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Comparison of the immunological activities of arabinoxylans from wheat bran with alkali and xylanase-aided extraction

Sumei Zhou[a,1], Xiuzhen Liu[c,1], Yan Guo[b], Qiang Wang[a], Daiyin Peng[c], Li Cao[b,*]

[a] Institute of Agro-food Science & Technology, Chinese Academy of Agricultural Sciences, Beijing 100193, China
[b] Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100193, China
[c] Anhui University of Traditional Chinese Medicine, Hefei, Anhui Province 230038, China

ARTICLE INFO

Article history:
Received 2 January 2010
Received in revised form 15 March 2010
Accepted 23 March 2010
Available online 30 March 2010

Keywords:
Wheat bran arabinoxylan
Alkaline extraction
Enzyme extraction
Immunomodulators

ABSTRACT

Differences in immunoregulatory activities of arabinoxylans (AXs) from wheat bran extracted by alkaline- and enzyme-based methods are unknown. We extracted AX from wheat bran by the 2 procedures. Chemical analysis revealed that alkaline-extracted AX (AX_A) contained a lower amount of protein (4.10%) than enzyme-extracted AX (AX_E, 9.85%) and no ferulic acid (as compared with 43.5 mg/100 g in AX_E). AX_A consisted of a highly substituted population with a ratio of arabinose to xylose of 0.83 (AX_E: 0.56), meanwhile the weight-average molecular weight of AX_A (3.517×10^5 Da) was about 10 times that of AX_E (3.252×10^4 Da). Both AX_A and AX_E had potent stimulating effects on innate and acquired immune responses on oral administration in female BALB/c mice. AX_E showed higher macrophage phagocytosis and delayed hypersensitivity reaction than did AX_A, with no significant differences in enhancing lymphocyte proliferation. Whether extracted by alkaline- or enzyme-aided methods, the obtained AXs could be explored as potent natural immunomodulators.

1. Introduction

Arabinoxylans (AXs) are major dietary fiber components of many cereals such as wheat, rye, corn, barley, oat, rice, and sorghum (Fincher & Stone, 1986). They consist of backbone chains of β-(1-4)-linked D-xylopyranosyl residues to which α-L-arabinofuranose units are linked as side chains, so they are often named as pentosans. The degree and distribution of side chains are important factors in physiochemical properties of AX (Hopkins et al., 2003). Pharmaceutical applications of AX from cereal have been investigated extensively and shown to prevent lifestyle-related diseases, and it was proved that AX has great effect on stimulating bowel movement, reducing blood sugar level, and inhibiting elevated cholesterol level (Lopez et al., 1999; Lu, Gibson, et al., 2000; Lu, Walker, et al., 2000; Lu, Walker, Muir, & O'Dea, 2004). Recently, AX from rice bran, which was enzymatically modified with extract from *Hyphomycetes mycelia*, was found to have an immune-enhancing function and anti-tumor activities (Badr El-Din, Noaman, & Ghoneum, 2008; Ghoneum & Abedi, 2004; Ghoneuma & Gollapudib, 2003; McDermott, Richards, Thomas, Montgomery, & Lewith, 2006). A partially hydrolyzed AX from corn husk also showed the ability to increase immunopotentiating activity in mice (Ogawa, Takeuchi, & Nakamura, 2005).

Wheat bran is an abundantly available by-product of the wheat milling industry. Nearly 20 million tons of wheat bran are generated annually in China, most of which is used as low-value feed component. The major constituents of wheat bran are nonstarch polysaccharides (NSPs), about 46% in industrial bran, 64–69% of NSPs are AXs (Ralet, Thibault, & Della Valle, 1990). Extracting AX from wheat bran could improve the use and maximize the potential of bran.

As the main component of the cell wall, AX is difficult to extract from wheat bran. A large portion of AX in wheat bran cannot be dissolved in water. The extent of physical entanglement, covalent ester bonding between carboxyl groups of uronic acids and the hydroxyl groups of AX, as well as the formation of diferulic acid bridges between adjacent arabinoxylan chains, might be related to its solubility (Fincher & Stone, 1986; Gruppen, Hoffmann, Kormelink, Voragen, & Kamerling, 1992). Cellulose and lignin in bran may interfere with isolation of AX (Izydorczyk & Biliaderis, 1995).

The 2 common ways to extract AX from bran are use of alkaline solutions and enzymatic degradation. Use of alkaline solutions is efficient for extracting AX from cell wall materials. The solutions NaOH, KOH, and $Ca(OH)_2$ are frequently used (Benamrouche, Crônier, Debeire, & Chabbert, 2002; Bergmans, Beldman, Gruppen, & Voragen, 1996) and sometimes H_2O_2 is added (Maes & Delcour, 2001). However, alkaline solutions are violent and may break down some functional groups of AX, such as ferulic acid,

* Corresponding author. Tel.: +86 10 62829611; fax: +86 10 62899748.
E-mail address: lcao@implad.ac.cn (L. Cao).
[1] These authors contributed equally to this work.

0144-8617/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2010.03.040

therefore, and may influence their functional properties. The xylanase extraction method is commonly used to degrade AX. Xylanase can attack the AX backbone in a random manner, so the water-unextractable AX becomes soluble and extractable (Andersson, Eliasso, Mangala, Kamal-Eldin, & Åman, 2003; Swennen, Courtin, Lindemans, & Delcour, 2006).

So far, no information exists on the immunological activities of wheat bran AX. The functional properties of polysaccharides are related to AX structure; differences in structure might be related to different extraction procedures used to extract AX. The differences in structure and immunoregulatory activities of AXs extracted by alkaline- and enzyme-based methods are unknown. In this study, we extracted wheat bran AX by alkaline- and enzyme-based procedures and compared chemical characterization, structural features, and immunoregulatory activities with the 2 extractions *in vivo* and *in vitro*.

2. Materials and methods

2.1. Materials

Fresh wheat bran was supplied by Beijing Guchuan Flour Co. (Beijing, China) and stored at 4 °C. Xylanase (Pentopan Mono) was kindly provided by China branch of Novozyme Co. (Beijing, China). RPMI-1640 medium, penicillin, streptomycin and all other tissue culture reagents were from GIBCO/BRL Life Technologies (Grand Island, NY, USA). Fetal bovine serum (FBS) was from HyClone Laboratories (Logan, UT, USA). Ginseng polysaccharide (GP) was from Shanxi Pude Pharmacy Co. (Shanxi, China). Giemsa dye, MTT (3-(4,5-dimethylthylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), dimethylsulfoxide (DMSO), lipopolysaccharide (LPS), and concanavalin A (ConA) were from Sigma Chemical Co. (St. Louis, MO, USA). Sheep red blood cells (SRBCs) and chicken red blood cells (CRBCs) were aseptically collected from a normal healthy sheep or a chicken, respectively, and stored in Alsever's solution at 4 °C.

2.2. Pre-treatment of wheat bran

The wheat bran was soaked for 60 min with de-ionized water at 4 °C and passed through 200 mesh, then the moist material was washed with 5–6 volume (w/v) of water for 3 times to remove starch. Water was squeezed from the suspension of washed bran, which was heated at 50 °C for about 12 h (water content decreased to about 10%). The dried wheat bran was ground to powder by use of a hammer mill and passed through 60 mesh. The de-starched bran was stored at 4 °C.

2.3. Alkaline extraction of polysaccharides (AX_A)

For extraction of AX_A, 100 g dried powder of de-starched wheat bran was mixed with 1.5 l of 0.15N NaOH (including 0.5% H_2O_2, v/v) for 90 min at 80 °C, then the mixture was cooled to room temperature and centrifuged (5000 × *g*, 30 min). The supernatant was neutralized with 0.2N HCl to about pH 4.5, centrifuged (5000 × *g*, 30 min), concentrated to about 1/4 of the original volume under reduced pressure and precipitated by use of ethanol (the final concentration 65%), then centrifuged (5000 × *g*, 5 min, 4 °C). The sediments were dissolved in water and centrifuged (5000 × *g*, 30 min, 4 °C). The supernatant was precipitated by ethanol as above and freeze-dried. The obtained fraction was AX_A.

2.4. Enzymatic extraction of polysaccharides (AX_E)

For extraction of AX_E, 100 g of de-starched wheat bran was mixed with 1.2 l de-ionized water and adjusted to about pH 5.5 with 0.1N HCl, incubated with 150 units of xylanase at 60 °C for 2 h with continuous stirring, then boiled for 10 min to inactivate the enzyme. After a cooling to room temperature, the wheat bran suspension was centrifuged (5000 × *g*, 30 min), and the supernatant was concentrated and precipitated by ethanol as for AX_A. The extract was AX_E.

2.5. Chemical analysis

All chemical analyses were performed in duplicate, and average results were expressed on a moisture-free basis. Moisture, ash, protein, starch content of wheat bran, purified AX_A and AX_E fractions were determined by ICC (International Association for Cereal Science and Technology; ICC, 1991) standard methods No. 109/1, 104/1, 105/2 and the AOAC (Association of Analytical Communities) Official Method 996.11 (Starch (Total) in Cereal Products). Pentosan content was estimated by the orcinol–hydrochloric acid method (Delcour, Vanhanel, & Geest, 1989). The ferulic acid content was measured on spectrophotometry at 375 nm (Izydorczyk, Biliaderis, & Bushuk, 1990), and lignin content was determined as acid-insoluble Klason lignin by the TAPPI (Technical Association of the Pulp and Paper Industry) standard method (T 222 om-06). Uronic acid content was determined by the m-phenylphenol method using glucuronic acid as standard (Blumenkrantz & Asboe-Hansen, 1973).

2.6. Analysis of sugar composition

Polysaccharides (AXA and AXE) were hydrolyzed in 1 M H_2SO_4 solution under 100 °C for 3 h, cooled to room temperature, then neutralized with $BaCO_3$. The sugar composition of AXA and AXE was determined as monosaccharide by high-performance liquid chromatography (HPLC) on a Waters system (Waters, USA) with a Sugar pak1 column (6.8 mm × 300 mm, SHODEX Co., Japan) and a differential refractive detector. After filtration (0.45 μm), the hydrolyzed samples (10 μl) were eluted with pure water at 0.5 ml/min and at 30 °C. The column was calibrated and the response factors were calculated by injecting a sugar standard mixture containing arabinose, xylose, glucose, galactose, and mannose (purity, 99%, Sigma–Aldrich, Inc., USA). Sugars present in the samples were identified by comparing their relative retention times with standards and were quantified by response factors. All analyses were conducted in duplicate, and values were required to be within a 5% reproducibility range.

2.7. Measurement of molecular weight

Average molecular weights and molecular weight distributions were determined by high-pressure size-exclusion chromatography with online multi-angle laser light scattering (DAWN-EOS, Wyatt Technology Inc., USA) with a K5 cell and a He–Ne laser (λ = 690 nm) and differential refractive index (DRI) detector (OPTILAB DSP). The column (TSK-gel G 5000, TOSOH, Japan) was eluted with, pH 7.2, 0.1 M phosphate buffer at 0.5 ml/min. The samples were filtered through a 0.45-μm filter. The collected data were analyzed by use of Astra software (Version 4.90.04, Wyatt). The concentration of each eluted fraction was determined by DRI (ERC 7515 A) according to the known value of dN/dC = 0.15.

2.8. Animals and their treatment with AX_E and AX_A

Female BALB/c mice (grade II, 5 weeks old) weighing 16 ± 2 g were purchased from the Experimental Animal Center of the Chinese Academy of Medical Sciences (Certificate No. SCXK-2004-0001, Beijing, China). The animals were housed in specific pathogen-free conditions (12-h light/12-h dark photoperiod, 22 ± 1 °C, 55 ± 5% relative humidity). All mice were allowed to

acclimatize in our facility for 1 week before experiments. All experiments were carried out according to the P.R. China legislation on the use and care of laboratory animals, and were approved by the Bioethics Committee of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

AX_E and AX_A were freshly prepared, and LPS contamination was tested by *Limulus* amebocytes lysate (LAL) assay using an E-TOXATE® kit according to the manufacturer's instruction. The quantity of endotoxin in AX_E and AX_A was less than 0.015 EU/mg (negative). Mice were divided randomly into 6 groups for treatment each day for 15 days ($n = 10$): AX_E or AX_A (100 and 200 mg/kg body weight for both, and in a volume of 0.1 ml/10 g body weight, orally or intraperitoneally); GP, 50 mg/kg body weight, intraperitoneally in a 0.1 ml/10 g body weight; and control, the same volume of 0.9% normal saline. All mice were intraperitoneally challenged with 0.2 ml of 0.1% SRBC on day 10. Mice were sacrificed by cervical dislocation after the last dose.

2.9. Delayed-type hypersensitivity reaction (DTH) by foot-pad measurement

At day 14, mice were primed with 2×10^8 SRBC suspensions in the right hind-foot-pad and PBS in the contralateral paw, and the thickness of the foot-pad was measured 24 h after SRBC challenge by use of a vernier caliper.

2.10. Preparation of spleen cell suspension

The mice were sacrificed, and spleens were removed aseptically. A single-cell suspension was prepared after cell debris and clumps were removed. Erythrocytes were depleted with ammonium chloride buffer solution. Lymphocytes were washed twice with PBS (phosphate buffered saline) containing 5% FBS and adjusted to 5×10^6 cells/ml in complete medium RPMI 1640 supplemented with 12 mM HEPES (4-(2-hydroxyerhyl) piperazine-1-erhanesulfonic acid, pH 7.1), 0.05 mM 2-mercaptoethanol, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% FBS.

2.11. Splenocyte proliferation assay in vivo

Splenic lymphocytes prepared from treated mice were cultured in triplicate wells of 96-well flat-bottom plates (5×10^5 cells/well) with ConA (2.5 μg/ml) or LPS (10 μg/ml) (Sigma, USA). After 44-h incubation at 37 °C with 5% CO_2, 20 μl MTT (5 mg/ml, Sigma) was added into each well for another 4-h incubation; plates were centrifuged ($1400 \times g$, 5 min) and the untransformed MTT was removed by pipetting. DMSO (200 μl) working solution (192 μl DMSO with 8 μl 1N HCl) was added to each well, and the absorbance was evaluated on a microtiter plate reader (Bio-Tek MQX200, USA) at 570 nm.

2.12. Splenocyte proliferation assay in vitro

Splenic lymphocytes from normal mice prepared as described previously were mixed with AX_E or AX_A (16, 32, 63, 125, 250, 500, 1000 μg/ml final concentration) and incubated with ConA (2.5 μg/ml) or LPS (10 μg/ml) as described previously. Absorbance at 570 nm was measured. The control experiments were performed without AX. All experiments were performed 3 times independently.

2.13. Phagocytosis of peritoneal macrophages

Phagocytosis of mice was detected as described (Yang et al., 2009). Briefly, mice were euthanized by cervical dislocation, and peritoneal macrophages were septically prepared from peritoneal exudates of mice after stimulation by intraperitoneally injection of 4 ml Hanks solution. FBS was added in the macrophage solution at a final titer of 20% (v/v). An equal volume of 1% (v/v) CRBCs was added. The mixture was then spread onto glass slides and incubated at 37 °C for 20 min in a humidified atmosphere. During the culturing period, the CRBCs were swallowed by the macrophages. Supernatants were then gently removed with use of a 0.9% NaCl solution, and the adherent cells were stained with Giemsa dye. The number of macrophage-ingesting CRBCs in at least 100 cells was calculated by direct visual enumeration on light microscopy. The phagocytic rate (PR) was calculated as follows:

$$\mathrm{PR}\ (\%) = \left(\frac{\text{number of macrophage} - \text{ingesting CRBCs}}{\text{total number of macrophages}}\right) \times 100.$$

Table 1
Yields and compositions of AXs extracted from de-starched wheat bran.

	De-starched wheat bran	AX_A	AX_E
Yield (%)[a]		18.5	12.4
Composition			
Protein (%)	18.4	4.1	9.8
Starch (%)	4.2	n.d.	n.d.
Pentosan (%)	39.8	88.9	85.6
Arabinose (%)[b]	25.0	41.8	34.8
Xylose (%)[b]	28.9	50.5	62.4
Glucose (%)[b]	43.9	7.7	2.8
Galactose (%)[b]	2.2	n.d.	n.d.
Mannose (%)[b]	n.d.	n.d.	n.d.
A/X[c]	0.86	0.83	0.56
Ferulic acid (mg/100 g)	–	n.d.	43.5
Uronic acid (%)	2.5	2.6	3.1
Lignin (%)	8.6	9.1	4.3
Ash (%)	6.7	0.5	0.1

All values were determined in duplicate. n.d., not detectable.
[a] Yield (%) was calculated as weight percentage of pentosan in obtained AXs based on de-starched wheat bran (dry basis).
[b] Expressed as weight percentage (%) of total sugar content.
[c] A/X: arabinose to xylose ratio.

2.14. Statistical analysis

Data are expressed as means ± SD and were analyzed with SPSS (version 12.0 for Windows, SPSS Inc.). Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Isolation and characterization of AX_E and AX_A

Commercial wheat bran contains some amounts of starch and protein, as well as pentosan (15–30% content on a dry matter basis), which may interfere with the extraction of AX. Because a granule of starch is smaller than that of bran, pre-treating bran by washing and wet-sieving (200 mesh) effectively removed starch; the amount of starch was decreased from 25.9% to 4.2% (Table 1). On the other hand, the content of pentosan was increased from 24.3% to 39.8%. Isoelectric precipitation at pH 4.5 was introduced to remove protein from AX. The chemical composition, as well as yield of AXA and AXE are shown in Table 1.

Compared with the yield of AX_E, that of AX_A was almost 50% greater (18.5% vs. 12.4%, Table 1). Therefore, alkaline solution (0.15 M NaOH containing 0.5% H2O2) was more effective in extracting AX from wheat bran than was xylanase hydrolysis. It is well known that protein by amide bond and ferulic acid by ester bond are chemically associated with AX, respectively, which can be hydrolyzed by alkaline media (Fincher & Stone, 1986), AXA

contained less protein (4.10%) than did AXE and almost no ferulic acid. Uronic acid contents showed no significant difference between the two AX products ($p < 0.05$).

The monosaccharide compositions of wheat bran and AXs were determined by HPLC. Both of the AXs contained more than 90% arabinose and xylose relative to total sugar (Table 1), whereas there were only 53.9% in wheat bran. More than 80% of glucose (mostly from cellulose in wheat bran) was removed after the two extraction procedures. AX_A contained more glucose than AX_E. But other minor neutral sugars, such as galactose and mannose which were found in previous reports (Hollmann & Lindhauer, 2005; Maes & Delcour, 2002) were not determined in this study. According to the latest report made by Revanappa, Nandini, and Salimath (2010), galactose and mannose were not detected in purified pentosans from whole wheat flour, which agreed with our results. Therefore, the contents of those minor sugars might be related to the purity of the extracts. As to the substituted degree or branch of arabinose, AXA contained a highly substituted population, with a ratio of A to X of 0.83, whereas that for AXE was only 0.56, for a low substituted population. The results are similar to those for water-unextractable and -extractable AXs in wheat bran (Maes & Delcour, 2002).

The elution profiles of AX_A and AX_E are in Fig. 1. Based on differential refractive index (DRI) signals, both AX_A and AX_E contained more than 1 elution peaks. Combined with laser light scattering signals, the low-molecular-weight peaks eluting after 22.0 min were detected for AX_A and AX_E samples. According to the detection limits of MALLS (10^3 to 10^8 Da), the molecular weight of the last peak was around or below to 10^3 Da. As the AXs were obtained by 65% (v/v) ethanol precipitation, oligosaccharide with low-molecular-weight within the supernatants was removed. However, there were certain amounts of lignin in AXs by composition analysis, especially in AX_A (Table 1), it implied that the substances of low-molecular-weight might be lignin.

The main peak of AX_A eluting at 16.2 min, which was earlier than that of AX_E (18.5 min), indicated that AX_A has a higher average molecular weight than AX_E. Astra analysis revealed that AX_A contained two populations with weight-average molecular weights of 7.233×10^6 and 3.517×10^5 Da (Table 2), with the content of the former population much lower; the calculated mass determined by the DRI was about 3% of the latter population (5.9605×10^{-6} to 1.7798×10^{-4} g). The weight-average molecular weight of the major population of AX_A was 10 times that of AX_E (3.252×10^4 Da). Values of Mw/Mn and Mz/Mn revealed that the polydispersity of AX_A was much larger than that of AX_E (Table 2), so obtaining AXE by the enzyme-extraction method was relatively uniform and produced a low degree of polymerized AX.

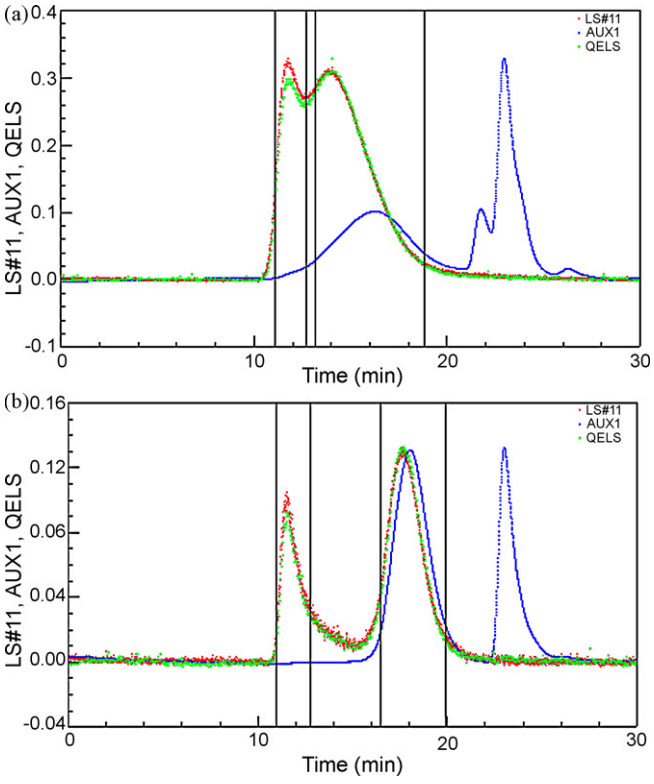

Fig. 1. High-performance size-exclusion chromatograms of AXs from wheat bran determined by differential refractive detector and multi-angle laser light scattering (curve in blue—differential refractive detector signal, curve in red—90° angle laser light scattering signal, and curve in green—quasi-elastic light scattering signal). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 3
Effect of oral treatment of AX_A and AX_E on sheep red blood cell (SRBC)-induced delayed-type hypersensitivity reaction in mice.

Group ($n = 10$)	Dosage (mg/kg/day)	Difference in paw swelling, mean ± SD
Control	0	0.015 ± 0.004
GP	50	0.040 ± 0.009[a]
AX_A	200	0.028 ± 0.007[a]
AX_A	100	0.020 ± 0.007[a]
AX_E	200	0.036 ± 0.006[a,c]
AX_E	100	0.025 ± 0.005[a,b]

GP = ginseng polysaccharide. a: $p < 0.001$ compared with control. b,c: $p < 0.05$ compared with AX_A (100 and 200 mg/kg, respectively).

3.2. Immunological activity of AX_E and AX_A

The DTH reaction is a cell-mediated pathologic response involved in T-cell activation and the production of many cytokines (Black, 1999) and an important index of overall immune function. Results of orally treating mice with AX_E and AX_A on SRBC-induced DTH reactions are in Table 3. The hypersensitivity response, characterized by foot-pad swelling, was evident 24 h after injection with a suspension of 2×10^8 SRBC into the right hind paw. Ginseng polysaccharide (GP), AX_E, and AX_A could significantly enhance the foot-pad swelling as compared with control treatment ($p < 0.001$), and AX_E showed higher DTH reactions than did AXA at 100 and 200 mg/kg ($p < 0.05$). However, intraperitoneally treating mice with AX_E and AX_A had no effect on DTH reactions (data not shown).

Lymphocyte proliferative responses are often used to evaluate the functional capacity of T- and B-lymphocyte immunity. ConA stimulates T-cell and LPS stimulates B-cell proliferation (Dai,

Table 2
Weight-average molecular weight (Mw), number-average molecular weight (Mn), *Z*-value molecular weight (Mz) and polydispersity (Mw/Mn, Mz/Mn) of AXs from wheat bran.

	Calculating mass (g)	Mw	Mn	Mz	Mw/Mn	Mz/Mn
AX_A	5.9605×10^{-6}	7.233×10^6 (1.0%)[a]	5.199×10^6 (1.1%)	1.113×10^7 (2.6%)	1.391 ± 0.021 (1.5%)	2.140 ± 0.060 (2.8%)
	1.7798×10^{-4}	3.517×10^5 (0.3%)	1.376×10^5 (0.6%)	7.008×10^5 (0.8%)	2.557 ± 0 .017 (0.7%)	5.094 ± 0.049 (1.0%)
AX_E	3.4906×10^{-4}	3.252×10^4 (1.6%)	3.171×10^4 (1.7%)	3.252×10^4 (1.6%)	1.025 ± 0.024 (2.3%)	1.053 ± 0.042 (4.0%)

[a] Percentages in ( ) indicates the reliability of values within 5%. All values were determined in duplicate.

Table 4
Effects of oral treatment of AX_A and AX_E on splenic lymphocyte proliferation in mice.

Group ($n = 10$)	Dosage (mg/kg/day)	A 570 nm OD, mean ± SD	
		LPS	ConA
Control	/	0.3958 ± 0.0497	0.4350 ± 0.0609
GP	50	0.5123 ± 0.0672[b]	0.5819 ± 0.0796[b]
AX_A	200	0.5045 ± 0.0690[b]	0.5193 ± 0.0779[b]
AX_A	100	0.4465 ± 0.0605	0.4915 ± 0.0361[a]
AX_E	200	0.4905 ± 0.0519[b]	0.5454 ± 0.0658[b]
AX_E	100	0.4397 ± 0.0661	0.5016 ± 0.0668[a]

OD = optical density; LPS = lipopolysaccharide; ConA = concanavalin A.
a,b: $p < 0.05$, $p < 0.01$ compared with control.

Table 5
Effects of AX_A and AX_E on phagocytic function of macrophages in mice abdominal cavities.

Group ($n = 10$)	Dosage (mg/kg/day)	Phagocytic rate (PR), mean ± SD
Control	/	17.2 ± 3.04
GP	50	29.9 ± 6.69[b]
AX_A	200	21.3 ± 4.42[a]
AX_A	100	17.3 ± 2.79
AX_E	200	26.3 ± 5.85[b,c]
AX_E	100	20.8 ± 3.58[a,d]

a,b: $p < 0.05$, $p < 0.01$ compared with control.
c,d: $p < 0.05$ compared with AX_A (100 and 200 mg/kg, respectively).

Zhang, Zhang, & Wang, 2009). Proliferation assay revealed that orally treating mice with AX_E and AX_A significantly increased ConA- and LPS-stimulated splenocyte proliferation at 100 and 200 mg/kg ($p < 0.05$, $p < 0.01$), and GP treatment (50 mg/kg) also had a strong effect on splenic lymphocyte proliferation as compared with control treatment ($p < 0.01$) (Table 4). AX_E and AX_A did not differ in induced lymphocyte proliferative response. However, *in vitro* assay or intraperitoneal treatment with AX_E and AX_A revealed no significant changes in ConA- and LPS-stimulated splenocyte proliferation (data not shown).

Activated macrophages participate in both specific and non-specific immune reactions but also are the "bridge cell" of these two kinds of immune reactions (Chen et al., 2008). Table 5 shows the phagocytic activity of macrophages isolated from mice orally treated with AX_E and AX_A. AX_E, AX_A, and GP enhanced phagocytosis by peritoneal macrophages as compared with control treatment; the PR was elevated significantly with AX_E (100 and 200 mg/kg) and AX_A (200 mg/kg). The phagocytic activity of AX_E was higher than that of AX_A at 100 and 200 mg/kg ($p < 0.05$). However, intraperitoneal treatment with AX_E and AX_A had no effect on enhancing phagocytosis (data not shown).

In summary, oral administration of alkaline- and enzyme-extracted wheat bran AX to mice both had a potent effect on innate and acquired immune responses. However, neither intraperitoneal treatment nor assay *in vitro* of AX_E and AX_A conferred any inmunostimulating effect. These results suggest the immunostimulating activity of wheat bran AX may be related to its metabolism *in vivo*. AX of wheat bran AX can be used as fermentable substrates for the growth of probiotic microorganisms present in the colon, such as lactobacilli and bifidobacteria, and act as a prebiotic. These probiotic microorganisms are induced by AX and synthesize extracellular hydrolytic enzymes such as xylanase and ferulic acid esterase, which can degrade AX (Grootaert et al., 2009; Vardakou, Nueno Palop, Gasson, Narbad, & Christakopoulos, 2007). Integrative systemic metabolic and microbiome profiling revealed that probiotics and prebiotics may have systemic effects on the host immune system and metabolism. Probiotics can stimulate immunity by increasing mucosal antibody production, thus boosting pro-inflammatory cytokine expression and enhancing host defensin production. Prebiotics can selectively stimulate beneficial microbes within the gut microbiota and may directly stimulate the immune system and enhance host defenses. The end-products of fermentation of prebiotics may be short-chain fatty acids (SCFAs) such as butyrate, which are able to improve mucosal morphology by increasing mucin production and decreasing translocation by binding to SCFA receptors on immune cells within the gut lymphoid-associated tissue (Saulnier, Spinler, Gibson, & Versalovic, 2009). However, further investigation about the relationship between the immunostimulating mechanisms of wheat bran AX and its function as a prebiotic is needed.

We also showed that the phagocytic activity of AX_E on macrophages and DTH reaction with oral administration to mice were higher than AX_A. The immune-enhancing function of wheat bran AX is related to molecular weight, chemical composition, and substituted degree or branch of arabinose. In this study, AX_E had lower average molecular weight and smaller polydispersity, as well as a low substituted population, and contained more protein and ferulic acid than did AX_A. Dietary supplementation rich in polyphenols such as ferulic acid, gallic acid, and catechin appears to enhance immune cell functions (Alvarez et al., 2006), so ferulic acid may be responsible for part of the higher immunostimulating activity of AX_E. Further studies are needed to elucidate the structure, function, relations and mechanisms responsible for wheat bran AX immunological activities.

4. Conclusions

AX extracted from wheat bran by alkaline and enzymatic methods had potent effects on innate and acquired immune responses in mice. Although the immune-enhancing activity of AX_E was higher than that of AX_A, the yield of AX_A was about 50% more than that of AX_E. AX extracted from wheat bran by alkaline or enzyme-aided procedures all could be explored as a good source of natural immunomodulator.

Acknowledgements

This work was supported in part by Basic Scientific Research Funds of State-level Public Welfare Scientific Research Institutes of the Chinese Academy of Agriculture Sciences and the Comprehensive Technology Platform of New Drug Research of the Ministry of Science and Technology (2009ZX09301-003).

References

Alvarez, P., Alvarado, C., Puerto, M., Schlumberger, A., Jiménez, L., & De la Fuente, M. (2006). Improvement of leukocyte functions in prematurely aging mice after five weeks of diet supplementation with polyphenol-rich cereals. *Nutrition*, *22*, 913–921.

Andersson, R., Eliasso, C., Mangala, S., Kamal-Eldin, A., & Åman, P. (2003). Effect of endo-xylanase-containing enzyme preparations and laccase on the solubility of rye bran arabinoxylan. *Journal of the Science of Food and Agriculture*, *83*, 617–623.

Badr El-Din, N. K., Noaman, E., & Ghoneum, M. (2008). In vivo tumor inhibitory effects of nutritional rice bran supplement MGN-3/Biobran on Ehrlich carcinoma-bearing mice. *Nutrition and Cancer*, *60*, 235–244.

Benamrouche, S., Crônier, D., Debeire, P., & Chabbert, B. (2002). A chemical and histological study on the effect of (1 → 4)-β-endo-xylanase treatment on wheat bran. *Journal of Cereal Science*, *36*, 253–260.

Bergmans, M. E. F., Beldman, G., Gruppen, H., & Voragen, A. G. J. (1996). Optimisation of the selective extraction of (glucurono) arabinoxylans from wheat bran: Use of barium and calcium hydroxide solution at elevated temperatures. *Journal of Cereal Science*, *23*, 235–245.

Black, C. A. (1999). Delayed type hypersensitivity: Current theories with an historic perspective. *Dermatology Online Journal*, *5*(1), 7. http://dermatology.cdlib.org/DOJvol5num1/reviews/black.html

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitive determination of uronic acids. *Analytical Biochemistry*, *54*, 484–489.

Chen, X. M., Lu, J. X., Zhang, Y. D., He, J. T., Guo, X. Z., Tian, G. Y., et al. (2008). Studies of macrophage immuno-modulating activity of polysaccharides isolated from Paecilomyces tenuipes. *International Journal of Biological Macromolecules*, *43*, 252–256.

Dai, Z. Y., Zhang, H., Zhang, Y. P., & Wang, H. H. (2009). Chemical properties and immunostimulatory activity of a water-soluble polysaccharide from the clam of *Hyriopsis cumingii* Lea. *Carbohydrate Polymers*, *77*, 365–369.

Delcour, J. A., Vanhanel, S., & Geest, C. D. (1989). Physico-chemical and functional properties of rye nonstarch polysaccharides. I. Colorimetric analysis of pentosans and their relative monosaccharides composition in fractional (milled) rye products. *Cereal Chemistry*, *6*, 107–111.

Fincher, G. B., & Stone, B. A. (1986). Cell walls and their components in cereal grain technology. In Y. Pomeranz (Ed.), *Advances in cereal science and technology* (pp. 207–295). St Paul: American Association of Cereal Chemists Inc.

Ghoneum, M., & Abedi, S. (2004). Enhancement of natural killer cell activity of aged mice by modified arabinoxylan rice bran (MGN-3/Biobran). *The Journal of Pharmacy and Pharmacology*, *56*, 1581–1588.

Ghoneuma, M., & Gollapudib, S. (2003). Modified arabinoxylan rice bran (MGN-3/Biobran) sensitizes human T cell leukemia cells to death receptor (CD95)-induced apoptosis. *Cancer Letters*, *201*, 41–49.

Grootaert, C., Van den Abbeele, P., Marzorati, M., Broekaert, W. F., Courtin, C. M., Delcour, J. A., et al. (2009). Comparison of prebiotic effects of arabinoxylan oligosaccharides and inulin in a simulator of the human intestinal microbial ecosystem. *FEMS Microbiology Ecology*, *69*, 231–242.

Gruppen, H., Hoffmann, R. A., Kormelink, F. J. M., Voragen, A. G., & Kamerling, J. P. (1992). Characterisation by ^{1}H NMR spectroscopy of enzymically derived oligosaccharides from alkali-extractable wheat-fluor arabinoxylan. *Carbohydrate Research*, *233*, 45–64.

Hollmann, J., & Lindhauer, M. G. (2005). Pilot-scale isolation of glucuronoarabinoxylans from wheat bran. *Carbohydrate Polymers*, *59*, 225–230.

Hopkins, M. J., Englyst, H. N., Macfarlane, S., Furrie, E., Macfarlane, G. T., & McBain, A. J. (2003). Degradation of cross-linked and non-cross-linked arabinoxylans by the intestinal microbiota in children. *Applied and Environmental Microbiology*, *69*, 6354–6360.

ICC International Association for Cereal Science and Technology. (1991). *Standard Methods*. Vienna: ICC International Association for Cereal Science and Technology.

Izydorczyk, M. S., & Biliaderis, C. G. (1995). Cereal arabinoxylans: Advances in structure and physicochemical properties. *Carbohydrate Polymers*, *28*, 33–48.

Izydorczyk, M. S., Biliaderis, C. G., & Bushuk, W. (1990). Oxidative gelation studies of water-soluble pentosans from wheat. *Journal of Cereal Science*, *11*, 153–169.

Lopez, H. W., Levrat, M. A., Guy, C., Messager, A., Demigne, C., & Remesy, C. (1999). Effects of soluble corn bran arabinoxylans on cecal digestion, lipid metabolism, and mineral balance (Ca, Mg) in rats. *Journal of Nutrition Biochemistry*, *10*, 500–509.

Lu, Z. X., Gibson, P. R., Muir, J. G., Fielding, M., & O'Dea, K. J. (2000). Arabinoxylan fiber from a by-product of wheat flour processing behaves physiologically like a soluble, fermentable fiber in the large bowel of rats. *Journal of Nutrition*, *130*, 1984–1990.

Lu, Z. X., Walker, K. Z., Muir, J. G., Mascara, T., & O'Dea, K. (2000). Arabinoxylan fiber, a byproduct of wheat flour processing, reduces the postprandial glucose response in normoglycemic subjects. *American Journal of Clinical Nutrition*, *71*, 1123–1128.

Lu, Z. X., Walker, K. Z., Muir, J. G., & O'Dea, K. J. (2004). Arabinoxylan fibre improves metabolic control in people with Type II diabetes. *European Journal of Clinical Nutrition*, *58*, 621–628.

Maes, C., & Delcour, J. A. (2001). Alkaline hydrogen peroxide extraction of wheat bran non-starch polysaccharides. *Journal of Cereal Science*, *34*, 29–35.

Maes, C., & Delcour, J. A. (2002). Structural characterisation of water-extractable and water-unextractable arabinoxylans in wheat bran. *Journal of Cereal Science*, *35*, 315–326.

McDermott, C., Richards, S. C., Thomas, P. W., Montgomery, J., & Lewith, G. (2006). A placebo-controlled, double-blind, randomized controlled trial of a natural killer cell stimulant (BioBran MGN-3) in chronic fatigue syndrome. *QJM*, *99*, 461–468.

Ogawa, K., Takeuchi, M., & Nakamura, N. (2005). Immunological effects of partially hydrolyzed arabinoxylan from corn husk in mice. *Bioscience, Biotechnology, and Biochemistry*, *69*, 19–25.

Ralet, M. C., Thibault, J. F., & Della Valle, G. (1990). Influence of extrusion-cooking on the physicochemical properties of wheat bran. *Journal of Cereal Science*, *11*, 793–812.

Revanappa, S. B., Nandini, C. D., & Salimath, P. V. (2010). Structural characterisation of pentosans from hemicellulose B of wheat varieties with varying chapati-making quality. *Food Chemistry*, *119*, 27–33.

Saulnier, D. M., Spinler, J. K., Gibson, G. R., & Versalovic, J. (2009). Mechanisms of probiosis and prebiosis: Considerations for enhanced functional foods. *Current Opinion in Biotechnology*, *20*, 135–141.

Swennen, K., Courtin, C. M., Lindemans, G. C. J. E., & Delcour, J. A. (2006). Large-scale production and characterisation of wheat bran arabinoxylooligosaccharides. *Journal of Science of Food Agriculture*, *86*, 1722–1731.

Vardakou, M., Nueno Palop, C., Gasson, M., Narbad, A., & Christakopoulos, P. (2007). In vitro three-stage continuous fermentation of wheat arabinoxylan fractions and induction of hydrolase activity by the gut microflora. *International Journal of Biological Macromolecules*, *41*, 584–589.

Yang, H. Y., Liu, S. L., Ibrahim, S. A., Zhao, L., Jiang, J. L., Sun, W. F., et al. (2009). Oral administration of live *Bifidobacterium* substrains isolated from healthy centenarians enhanced immune function in BALB/c mice. *Nutrition Research*, *29*, 281–289.