JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chemical, Molecular, and Structural Characterization of Alkali Extractable Nonstarch Polysaccharides from Job's Tears

SUPAPORN APIRATTANANUSORN,[†] SUNANTA TONGTA,*[,†] STEVE W. CUI,[‡] AND QI WANG[‡]

School of Food Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand 30000, and Guelph Food Research Centre, Agriculture and Agri-Food Canada, 93 Stone Road West, Guelph, Ontario, Canada N1G 5C9

The polished Job's tears (*Coix lachryma-jobi* L.) seeds, dark and white husk types, were sequentially extracted by hot water (75 °C) and 0.5 M NaOH solution. Nonstarch polysaccharides were not found in the water extract but were present in the alkali extract. The major components of the alkali extract from both Job's tears were protein, ash, and nonstarch polysaccharides, mainly arabinoxylans. The high arabinose to xylose ratio of 1.25 and 1.24 indicated a highly substituted structure. The average molecular weight (MW) of arabinoxylans of the dark and white husk types were 741,000 Da (Pd 1.5) and 1,449,000 Da (Pd 2.6), respectively, and their average MW reduced after treatment with protease. The alkali extractable arabinoxylans were elucidated to have a (1,4)-linked β-D-xylan main chain highly substituted with single arabinose units. The results showed that the α-L-arabinofuranosyl residues (Ara*f*) were attached to the main chain mostly at O-3, followed by both O-2 and O-3 of xylopyranosyl residues (Xyl*p*).

KEYWORDS: Job's tears; nonstarch polysaccharides; alkali extract; arabinoxylans

INTRODUCTION

Coix lachryma-jobi L., commonly named Job's tears, is a grain crop in the family of Gramineae. It is also known as adlay in the Philippines and as ma yuen in China, and is widely cultivated in many Asian countries such as the Philippines, Burma, China, Sri Lanka, and Thailand. Job's tears seed is pear-shaped, approximately 5 mm in diameter, and covered with a hard, shiny, dark brown to gray-black hull, which is dehulled during the milling process and is usually polished to obtain kernels before use. Job's tears has long been used in traditional Chinese medicine and as a nourishing cereal. It is added to soups and broths in the form of flour or whole grain. In Japan and Thailand, a nondairy drink from Job's tears is available in the market as alternatives for health benefit. The consumption of Job's tears has been demonstrated to have a number of beneficial biomedical functions such as antitumor, antiallergic, and fibrinolytic activities (*1*−*6*). Numata et al. (*6*) reported that an acetone extract of Job's tears seeds attributed to an acidic fraction had antitumor activity in tests in vivo. The methanolic extract from Job's tears exhibited the most capacity to reduce anti-OVA IgE (*3*) and inhibited the proliferation of tumor and cancer cells (*1, 2*). In addition, it was reported that a neutral lipid extract from the endosperm of Job's tears was effective against malignant tumors in Chinese patients (*7*).

Recently, animal and human clinical trials have shown that the consumption of Job's tears may potentially improve lipid metabolism, thus decreasing the risk of heart diseases (*8, 9*). However, the active components and associated mechanism are not clear. Yu et al. (*10*) reported that the water soluble polysaccharide enriched fraction of Job's tears lowered the level of serum total cholesterol, LDL cholesterol and triglyceride, in hamsters. Kim et al. (*11*) also reported that the levels of

Table 1. Chemical Composition of the Alkali Extracts from Job's Tears Before and After Protease Treatment

	dark husk type		white husk type	
composition (% dry weight basis)	before[a]	after[b]	before[a]	after[b]
protein	47.8 ± 0.19	21.4 ± 0.30	38.4 ± 0.98	22.4 ± 0.37
ash	24.4 ± 0.03	20.8 ± 0.03	30.1 ± 0.13	25.5 ± 0.22
uronic acid	2.1 ± 0.14	4.6 ± 0.19	1.7 ± 0.01	3.5 ± 0.02
total sugar	18.5 ± 0.55	n.d.	15.2 ± 0.54	n.d.
arabinose	6.6 ± 0.02	18.3 ± 0.05	5.1 ± 0.04	15.0 ± 0.09
xylose	5.3 ± 0.02	14.6 ± 0.00	4.1 ± 0.06	12.4 ± 0.13
glucose	4.9 ± 0.17	0.60 ± 0.02	5.6 ± 0.18	0.8 ± 0.05
galactose	1.0 ± 0.14	2.6 ± 0.07	0.8 ± 0.04	2.1 ± 0.10
mannose	0.2 ± 0.00	0.3 ± 0.10	0.2 ± 0.02	0.4 ± 0.01
Ara/Xyl	1.25	1.25	1.24	1.21

[a] The alkali extract before protease treatment. [b] The alkali extract after protease treatment. n.d., not determined; Ara/Xyl, arabinose-to-xylose ratio.

* Corresponding author. Tel: +66 44 224266. Fax: +66 44 224387. E-mail: s-tongta@g.sut.ac.th.
[†] Suranaree University of Technology.
[‡] Agriculture and Agri-Food Canada.

10.1021/jf801231y CCC: $40.75
Published on Web 08/22/2008

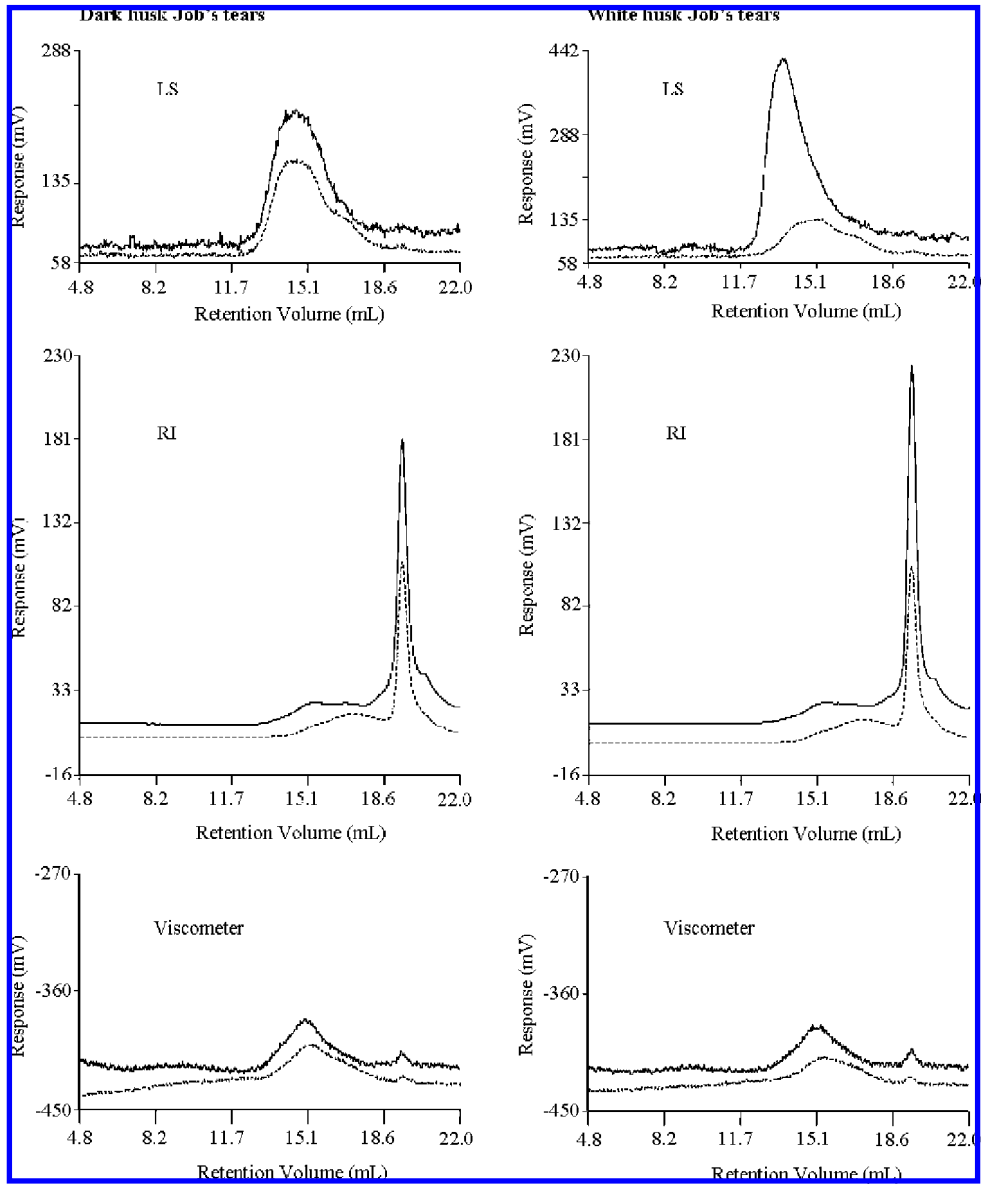

Figure 1. Elution profiles of the alkali extracts from Job's tears on two serially connected columns coupled with triple detectors. LS = light scattering detector; RI = refractive index detector; Viscometer = Viscosity detector. The solid line shows the alkali extract before protease treatment; the dashed line shows the alkali extract after protease treatment.

triglyceride, total cholesterol, and leptin in blood serum of rats were significantly decreased when they were fed a crude water extract of Job's tears. Water soluble β-glucans from cereals such as oats and barley have been well known to have such hypolipidemic effects (*12*). Similarly, arabinoxylans from corn bran were also reported to reduce cholesterol level (*13*). Limited information on nonstarch polysaccharides in Job's tears is currently available. Therefore, the objective of this study is to investigate the chemical and molecular properties of extracted nonstarch polysaccharides from Job's tears, which may be associated with biological properties.

MATERIALS AND METHODS

Materials. Two types of polished Job's tears seeds, with dark and white husks, grown in Thailand, were obtained from CCP Northern Co., Ltd., Thailand. Thermostable α-amylase 3,000 U/mL (45 U/mg) from *Bacillus licheniformis* was obtained from Megazyme International (Bray, Co. Wicklow, Ireland), and protease from *Streptomyces griseus* (4 U/mg) was obtained from Sigma-Aldrich. All chemicals used were of reagent grade.

Composition Analysis of Job's Tears. Moisture and ash were determined according to AOAC (*14*). Protein was analyzed using an NA2100 Nitrogen and Protein Analyzer/ThermoQuest Itallia SPA EA/NA 1110 Automatic Elemental Analyzer (Strada Rivoltana, Milan, Italy) with the factor of 6.25 to convert nitrogen to protein content. Lipid content was analyzed using a 2050 Soxtec Avanti autoextraction unit connecting with a 2050 Soxtec autocontrol unit and drive unit (Foss Tecator, Sweden). Total dietary fiber content was determined using the total dietary fiber assay kit from Sigma-Aldrich. The content of starch was determined with slight modification as described by Wood et al. (*15*). Total sugar content was determined according to the method of Dubois et al. (*16*).

Sample Preparation. Samples were cleaned to remove dust and other defects (such as small fractured husks) before grinding and passing through a 35-mesh sieve. The ground samples were refluxed with 70% EtOH at a ratio of 1:10. The ethanol extract was removed by filtration, and the residue was dried at 70 °C, overnight, then reground and passed a 35-mesh sieve. This sample was considered as residue A.

Extraction of Nonstarch Polysaccharides. Residue A was hydrolyzed by α-amylase at 90 °C for 30 min and at 75 °C for 150 min to remove starch and extract water extractable nonstarch polysaccharides. After filtration, the residue was washed with deionized water (75 °C,

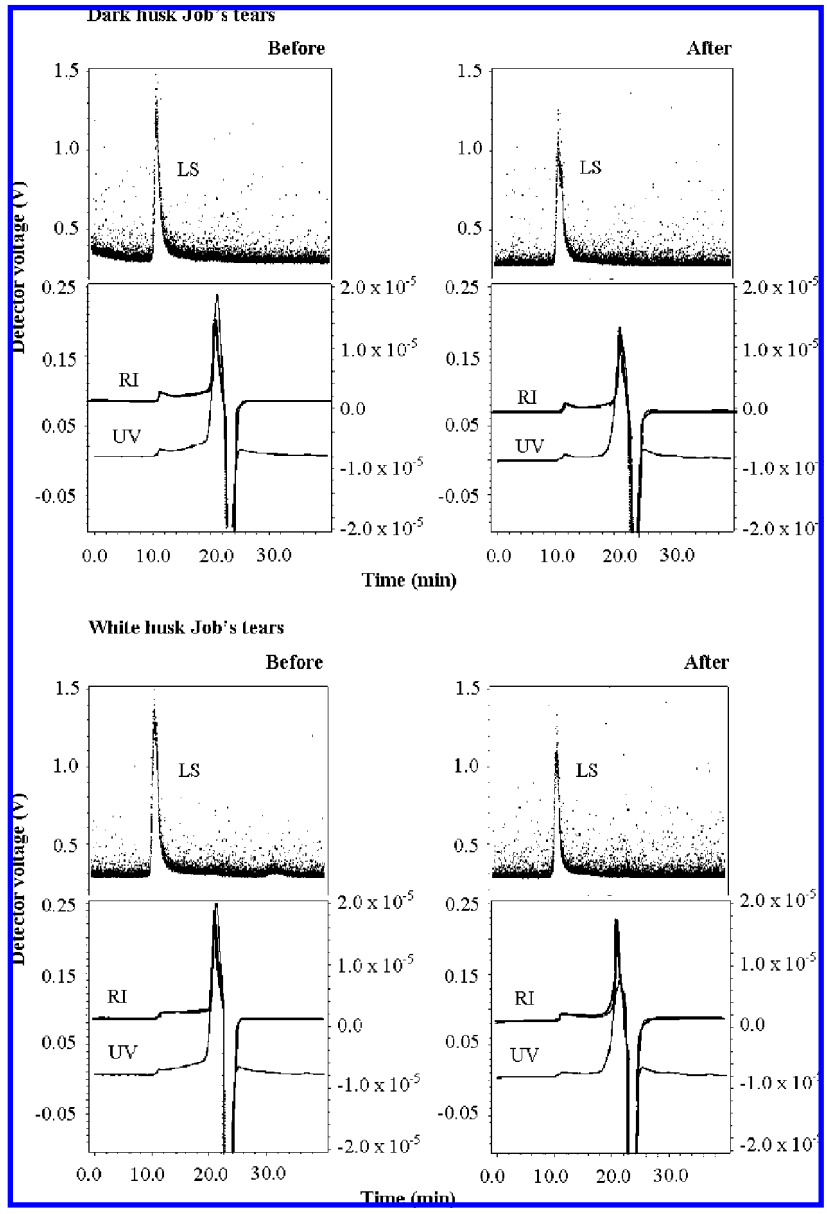

Figure 2. Elution profiles of the alkali extracts from Job's tears on a column coupled with triple detectors. LS = light scattering detector; RI = refractive index detector; UV = ultraviolet detector; Before = before protease treatment; After = after protease treatment.

15 min), filtered, and air-dried overnight. The dried sample was noted as residue B. The filtrates from the two steps were pooled and heated to 100 °C for 15 min to deactivate α-amylase, and the pH was adjusted to 4.5 with 2 M HCl for protein precipitation. Then, it was centrifuged at 10,000 rpm for 15 min to obtain the supernatant. After dialysis against deionized water for 2 days, the supernatant was concentrated by vacuum rotary evaporation and then freeze-dried. This dried sample was assigned as the water extract.

Residue B was extracted with 0.5 M NaOH at 25 °C for 3 h in order to extract alkali extractable nonstarch polysaccharides and then centrifuged at 10,000 rpm for 15 min to obtain the supernatant. The supernatant was adjusted to pH 4.5, and then it was centrifuged. The supernatant was dialyzed against water for 2 days, concentrated, and then freeze-dried. The dried sample was redissolved in water and adjusted to pH 7. After centrifugation, the supernatant was freeze-dried and designated as the alkali extract.

Determination of Uronic Acid, Mineral, and Amino Acid Composition. The uronic acid content was determined according to Blumenkrantz et al. (*17*). The mineral content was determined by atomic absorption spectroscopy (AAS), and the amino acid composition was determined according to AOAC 994.12 AA in Feeds and AOAC 985.28 Sulfur AA in Food and Feed Ingredient by Laboratory Service, University of Guelph, Canada.

Monosaccharide Analysis. Samples were hydrolyzed in 1 M H_2SO_4 at 100 °C for 2 h and diluted. The monosaccharide hydrolysates were filtered (0.45 μm) and the volume of 50 μL was injected onto a high performance anion exchange chromatography (HPAEC) system (Dionex, Sunnyvale, CA) using a 250 mm × 4.0 mm i.d. CaboPac PA1 column connected with pulsed amperometric detection (PAD). Gradient elution was carried out using 100 mM NaOH (A) and deionized water (B) from 8% A:92% B to 0% A:100% B over 45 min at a flow rate of 1.0 mL/min.

Molecular Size Distribution. The molecular weight of the alkali extract was determined using high performance size exclusion chromatography (HPSEC) coupled with triple detectors including refractive index, right angle laser light scattering (RALLS), and a model 250 dual viscosity detector (Viscotek, Houston, USA). The Shimadzu SCL-10Avp pump unit (Shimadzu Scientific Instruments Inc., Columbia, MD, USA) was equipped with two serially connected columns, a 300 mm × 8.0 mm i.d. Shodex OHpak SB-806 M HQ column (Showa Denko K.K., Tokyo, Japan) and a 300 × 7.8 mm i.d. Ultrahydrogel linear column (Waters, Milford, USA), which were maintained at 40 °C. The eluent was 100 mM sodium nitrate

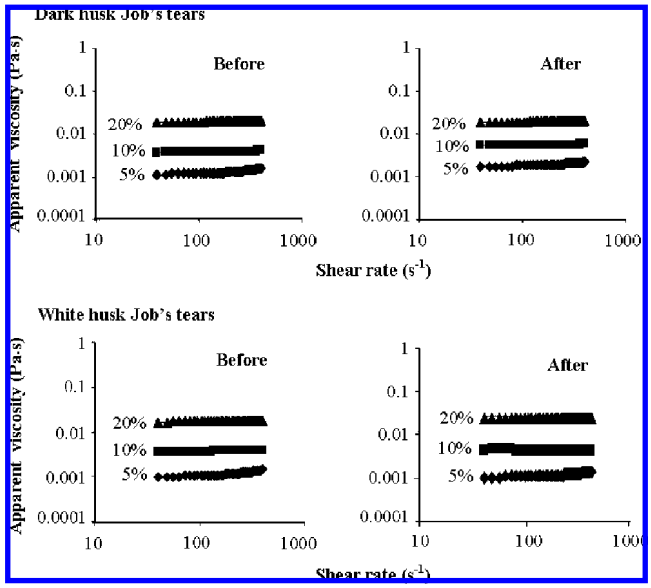

Figure 3. Steady shear rheological flow behavior of the alkali extracts from Job's tears. Before = before protease treatment; After = after protease treatment.

Table 2. Methylation Analysis and Mode of Linkage of the Alkali Extractable Nonstarch Polysaccharides from Job's Tears

methylated sugar	deduced linkage	molar ratio[a] dark husk type	molar ratio[a] white husk type
2,3,5-tri-*O*-methyl arabinitol	T-Ara*f*	3.6	3.1
2,3-di-*O*-methyl pentitol	1,4-Xyl*p*	1.0	1.0
2-*O*-methyl pentitol	1,3,4-Xyl*p*	2.4	3.7
1-deuterio pentitol pentaacetate	1,2,3,4-Xyl*p*	1.4	1.4
4-*O*-methyl pentitol	1,2,3-Xyl*p* (end)	0.7	0.2
2,4-di-*O*-methyl pentitol	1,3-Xyl*p* (end)	0.1	0.1
2,3,6-tri-*O*-methyl hexitol	1,4-Gal*p*	1.1	0.5
2,3,4-tri-*O*-methyl hexitol	1,6-Gal*p*	0.4	0.5
3,6-di-*O*-methyl hexitol	1,2,4-Gal*p*	0.5	0.3
2,3,4,6-tetra-*O*-methyl hexitol	1-Gal*p* (end)	0.4	0.1

[a] Relative molar ratio calculated from the ratio of the peak height and normalized to 1,4-Xyl*p*.

($NaNO_3$) containing 0.03% sodium azide (NaN_3) (0.6 mL/min). Pullulan standards P100, P400, and P800 were used for calibrating the detectors. With the triple detectors, it allows one to obtain the average molecular weight, polydispersity index (Pd), radius of gyration (Rg), and intrinsic viscosity ([η]) of the measured sample. Samples were dissolved in deionized water (5 mg/mL) at 50 °C for 3 h before the solution was filtered through a 0.45 μm filter, and a volume of 100 μL was then injected onto the column system. In addition, the sample was also monitored by a HPSEC system combining a laser light scattering detector (DAWN DSP-F, Wyatt Technologies, Santa Barbara, CA), a refractive index detector (Optilab DSP, Wyatt Technologies, Santa Barbara, CA) and a UV detector (Biosep-SEC-S3000, Phenomenex, Torrance, CA) in order to determine the fractions of polysaccharides and proteins. The aliquot of samples was filtered (0.45 μm) and injected onto a 300 mm × 10.0 mm i.d. Biosep 4000 column (Phenomenex), running by 100 mM $NaNO_3$ (pH 7) at 0.5 mL/min.

d*n*/d*c* Measurement. The refractive index increment (d*n*/d*c*) was determined with the BI-DNDC differential refractometer (Brookhaven, NY, USA) at 30 °C and λ_0 = 535 nm. The alkali extract was dissolved in 100 mM $NaNO_3$. The d*n*/d*c* value was determined from the slope of the plot of the refractive index against various concentrations, ranging from 2.0 to 0.2 mg/mL.

Intrinsic Viscosity Measurement. The alkali extract was dissolved in water or 0.05 M NaCl solution, and then measured at 23 ± 0.05 °C using a 50 mL Ubbelohde capillary viscometer (Cannon Instrument, PA, USA). The relative viscosity and specific viscosity against concentration were plotted and extrapolated to zero concentration. The intrinsic viscosities were then calculated according to the Huggins (*18*) and Kraemer (*19*) equations.

Surface Tension. The surface tension of the alkali extract was determined by a semiautomatic model 21 Surface Tensionmat tensiometer (Fisher Scientific, Toronto, Canada) at 23−24 °C. This tensiometer is based on the Du Nouy ring method to measure static surface tension. The various concentrations of solutions were placed in dishes with a diameter of 7.5 cm.

Rheological Measurement. The steady shear flow measurement of the alkali extract solution with concentrations of 5%, 10%, and 20% (w/v) was carried out using a Bohlin VOR rheometer. A cone and plate geometry with 50 mm diameter and 4° cone angle was used. The temperature was maintained at 23 °C during all measurements.

Protease Treatment. The alkali extract was dispersed in 0.1 M phosphate buffer at pH 7.5 and subjected to protease. The mixer was placed at 37 °C and incubated for 16 h. The enzyme was inactivated by heating at 100 °C for 15 min and precipitated by centrifugation at 10,000 rpm. The supernatant was dialyzed against deionized water for 2 days and then freeze-dried.

Structural Feature of Alkali Extractable Nonstarch Polysaccharides. The alkali extracts were dispersed in phosphate buffer and subjected to protease to extensively hydrolyze protein at 37 °C. The enzyme was inactivated by heating at 100 °C and precipitated by centrifugation (10,000 rpm). The supernatant was dialyzed against water for 2 days, concentrated, and precipitated by 100% EtOH, and then the residue was air-dried. The glycosidic linkages of dried samples were determined by methylation analysis using a modified method of Ciucanu et al. (*20*). The aliquot (1 μL) of partially methylated alditol acetates (PMAA) was injected onto the GC-MS system (ThermoQuest Finnigan, San Diego, CA). The 30 m × 0.25 mm, 0.2 μm SP-2330 fused silica column (Supelco, Bellefonte, PA) was interfaced with an ion trap MS detector. Helium with a flow rate of 1 mL/min was used as the gas carrier with the condition of 160−210 °C at a rate of 2 °C/min, and held at 210 °C for 8 min. The temperature was then increased to 240 °C at a rate of 5 °C/min and held for 6 min. In addition, the structural feature of samples was studied using NMR spectroscopy. The samples were dissolved in D_2O, freeze-dried three times, and finally dissolved in D_2O (4% w/v) before NMR analysis. The ^1H NMR and ^{13}C NMR spectra were recorded at 30 °C on a Bruker AMX-500 FT spectrometer. Frequency pulse angle 45° with presaturation was recorded for each sample. Tetramethylsilane (TMS) was used as an internal reference for both ^1H and ^{13}C NMR. Two-dimensional NMR (HMQC, COSY, TOCSY, and HMBC) was performed using the standard Bruker pulse sequence.

RESULTS AND DISCUSSION

Composition of Polished Job's Tears Seeds. Polished Job's tears contained a moisture of 9.6% and 9.3% for the dark and white types, respectively. The major composition of Job's tears was starch and protein, which were 67.5% and 15.5%, respectively, for the dark husk type and 69.4% and 13.6%, respectively, for the white one. Total dietary fiber was 17.4% in the dark husk Job's tears and 14.7% in the white husk Job's tears, which were similar to the other sources of cereal, barley, wheat, oats, corn, and sorghum (17.7, 14.9, 11.5, 13.1, and 11.5%, respectively) (*21*). There were small differences in fat and ash between the two types, which were 5.5−5.9% of fat and 1.6−1.7% of ash.

Composition of Water and Alkali Extracts. There were no water extractable nonstarch polysaccharides in the water extracts of residue A from both types of Job's tears. The water extracts were composed of only starch (83% dry weight), which remained after hydrolysis by α-amylase. To ensure the results, the water extract was run by HPAEC, and it was found that only glucose without other sugars was detected. Yamada et al. (*22*) found that the main water extractable polysaccharide in

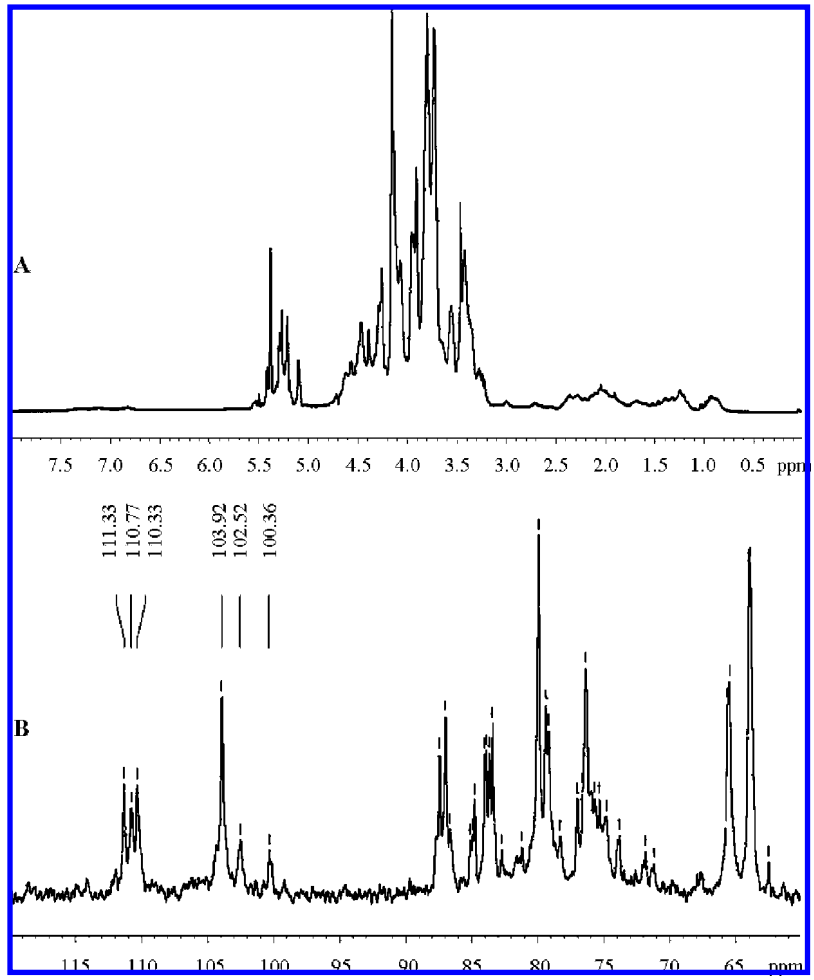

Figure 4. NMR spectra of alkali extractable arabinoxylans from Job's tears relative to internal TMS. (**A**) ^{1}H NMR. (**B**) ^{13}C NMR.

Job's tears seed was an amylopectin-like glucan containing (1,4)-linked α-D-glucose as a main chain in which the glucosyl residues were attached at O-6. The other polysaccharides consisting of rhamnose, arabinose, xylose, galactose, galacturonic acid, and glucuronic acid were also reported (*23*). For the present study, nonstarch polysaccharides, for example, the arabinoxylans were not able to be extracted by hot water, probably as a result of the physical entanglement and the degree and substitution pattern of themselves in the endosperm. The covalent ester bonds between ferulic acids and other components such as lignin in the cell wall matrix is also a possible explanation (*24*).

Residue B of the dark husk type contained 5.56% total sugar, 1.73% arabinose, 0.36% galactose, 2.13% glucose, 1.24% xylose, and 0.10% mannose and that of the white husk type contained 5.54% total sugar, 1.63% arabinose, 0.35% galactose, 1.85% glucose, 1.25% xylose, and 0.07% mannose. Residue B of both types contained starch less than 1%. After extraction by 0.5 M NaOH, protein was found to be the major component in the alkali extract of residue B from both dark (47.8%) and white husk (38.4%) Job's tears (**Table 1**). The proteins in the alkali extracts of both types have similar amino acid profiles, being high in glutamic acid and methionine sulfone, but low in cysteic acid, phenylalanine, and isoleucine. Interestingly, it was found that the alkali extract was very rich in ash content, 24.4% in the dark husk type and 30.1% in the white husk type. The alkali extract was high in phosphorus (6 and 8%) and calcium (3 and 4%). The majority of phosphorus in cereals was reported as being associated with phytic acid, which may occur in some regions of seeds as a mixed calcium−magnesium−potassium salt (*25*). The uronic acid content expressed as galacturonic acid equivalent was 2.1% and 1.7% in the dark and white husk types, respectively. Both alkali extracts from the dark and white husk Job's tears were composed of low content of total sugar, 18.5% and 15.2%, respectively.

The nonstarch polysaccharides of the alkali extract from the dark husk type were mainly composed of 6.6% arabinose, 5.3% xylose, and 4.9% glucose, followed by 1.0% galactose and 0.2% mannose (**Table 1**). Similarly, those of the white husk type were high in arabinose (5.1%), xylose (4.1%), and glucose (5.6%), followed by galactose (0.8%) and mannose (0.2%). The arabinose and xylose are considered to be the parts of arabinoxylans in cereals, possibly esterified with the ferulic acids in the cell wall of endosperm and released by hydrolysis with alkali (*26*). The ratio of arabinose to xylose (Ara/Xyl) was 1.25 in the dark and 1.24 in the white husk types, indicating a much higher degree of branching than that reported in the arabinoxylans of wheat (0.6−0.8) (*27*), barley (0.3−0.6) (*28*), and rye (0.2−0.6) (*29*). The galactose that was found in a less extent is possibly from arabinogalactan, which is also present in some cereals at a low level. Mannose might be derived from glucomannans, which have been found in various cereal cell walls (*30*).

Molecular Size Distribution and Intrinsic Viscosity ([η]). The size exclusion chromatograph profiles of the alkali extracts from both Job's tears detected by triple detectors are shown in **Figure 1**. Both refractive index (RI) and viscosity detectors presented two fractions of different size, while the light scattering (LS) detector showed only the high molecular weight (MW) fraction. This is due to the reason that the LS signal is less sensitive to small molecules. The elution volumes of fraction

Table 3. Chemical Shift (δ) Assignments of ^{1}H NMR and ^{13}C NMR Spectra of the Alkali Extractable Arabinoxylans from Job's Tears on the basis of HMQC, HMBC, COSY, and TOCSY

glucosyl residues	assigned H, C position[a]	^{1}H (ppm)[b]	^{13}C (ppm)[c]
α-L-Ara*f* O-3 linked	1	5.38	110.3
	2	3.90	79.9
	3	4.14	83.4
	4	4.25	87.4
	5-eq	3.77	65.4
	5-ax	3.72	
α-L-Ara*f* O-3 linked (also at O-2)	1	5.25	110.7
	2	4.15	84.7
	3	3.94	79.3
	4	4.38	83.4
	5-eq	3.79	64.0
	5-ax	3.71	
α-L-Ara*f* O-2 linked (also at O-3)	1	5.20	111.3
	2	4.13	87.0
	3	3.95	79.3
	4	4.20	—
	5-eq	3.81	64.0
	5-ax	3.72	
β-L-Ara*f*	1	5.10	103.9 region of Xyl*p* anomeric carbon
	2 (from COSY)	4.07	65.4
	—	4.12	-
	—	3.91	-
	—	3.81	65.4
	—	3.72	
β-D-Xyl*p* disubstituted	1	4.61	100.3
	2	not present in TOCSY	87.0 (from HMBC)
	3	not present in TOCSY	87.0 (from HMBC)
	—	not present in TOCSY	—
	—	not present in TOCSY	—
	—	not present in TOCSY	
β-D-Xyl*p* monosubstituted at O-3	1	4.58	102.5
	2	3.55	74.7
	3	3.64	87.4 (from HMBC)
	4	3.75	79.9
	5-eq	3.95	-
	5-ax	3.48	
β-D-Xyl*p* unsubstituted	1	4.46	103.9
	2	3.43	62.5
	3	3.65	—
	4	3.72	—
	5-eq	3.83	76.6
	5-ax	3.52	

[a] Compared to literature. [b] Assigned from ^{1}H NMR and TOCSY. [c] Assigned from ^{13}C NMR and HMQC unless indicated otherwise.

1 (high MW) and fraction 2 (low MW) for the dark husk Job's tears were approximately 14.0 and 19.0 mL, respectively, which were almost the same as those of the white husk Job's tears. After protease treatment, the peaks of high MW fraction shifted to a longer retention volume, indicating the reduction of average MW, especially for the white husk type sample. A similar result was obtained for an arabinoxylan−protein complex isolated from rye bran, where MW decreased after treating with Pronase (*31*). The average MW of fraction 1 was estimated to be 741,000 (Pd 1.5, Rg 44.5 nm, and [η] 3.1 dL/g) and 1,449,000 Da (Pd 2.6, Rg 52.3 nm, and [η] 3.5 dL/g) for the dark and white husk Job's tears, respectively, and reduced to 369,000 (Pd 2.7, Rg

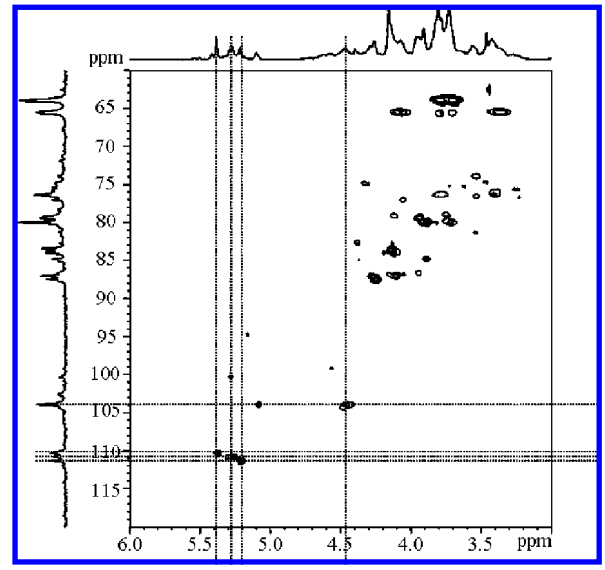

Figure 5. ^{1}H/^{13}C HMQC correlation of alkali extractable arabinoxylans from Job's tears.

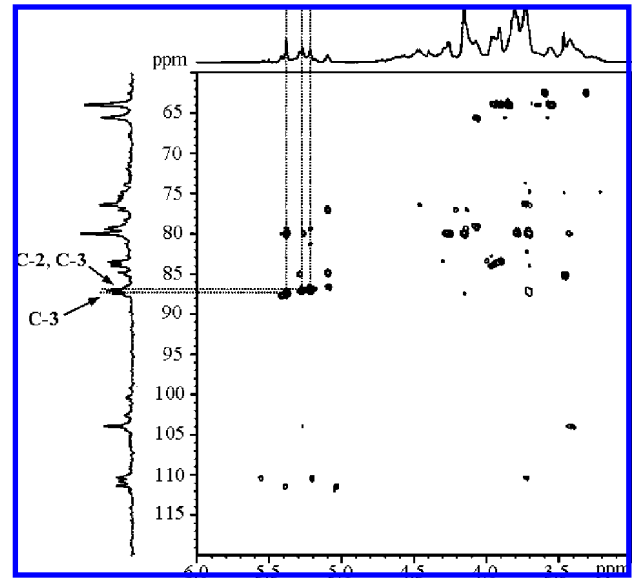

Figure 6. ^{1}H/^{13}C HMBC correlation of alkali extractable arabinoxylans from Job's tears.

27.2 nm, and [η] 2.2 dL/g) and 244,000 Da (Pd 1.6, Rg 22.9 nm, and [η] 1.7 dL/g) after protease treatment. The strong LS signal from the high MW fraction (fraction 1), which showed relatively low intensity for RI detector, was attributed to the highly branched arabinoxylans rather than protein polymers. The higher values of radius of gyration (Rg) and intrinsic viscosity ([η]) were consistent with the higher average MW of this fraction. The MW of fraction 2 could not be accurately calculated due to weak LS signals, but was estimated in the range of several thousand daltons.

To expand information on the protein and carbohydrate fractions, the alkali extracts of the dark and white husk types were analyzed using the Biosep 4000 column coupled with LS, RI, and ultraviolet (UV) detectors. The elution profiles from UV and RI detectors also contained two peaks (**Figure 2**) of different size, while light scattering only showed one peak corresponding to the high MW fraction. This suggests the presence of a protein component in both fractions and that the protein might be chemically associated with the carbohydrate polymers. The results were consistent with that from Storsley

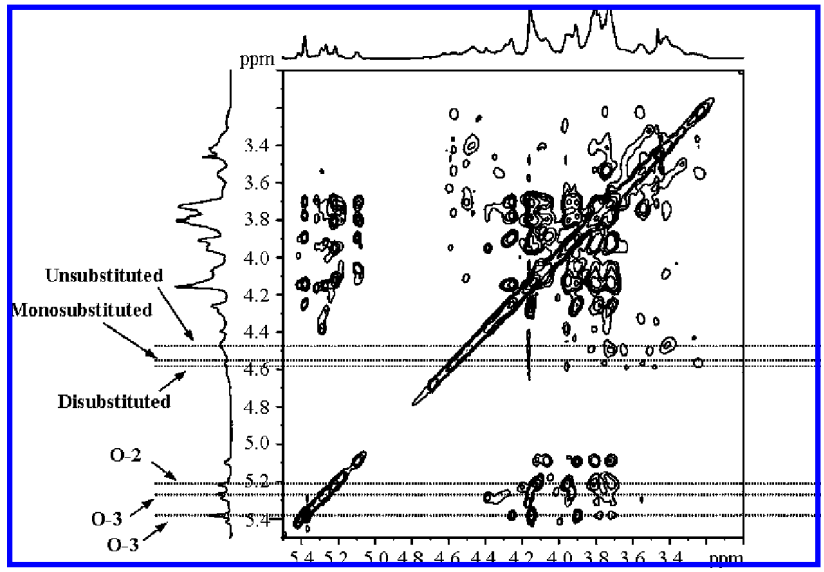

Figure 7. $^{1}H/^{1}H$ TOCSY correlation of alkali extractable arabinoxylans from Job's tears.

et al. (*32*) who reported that arabinoxylans were associated to some extent with protein. After protease treatment, the glucose and protein contents of the alkali extracts were obviously reduced, while their arabinose and xylose increased, and the ratio of arabinose to xylose remained the same (**Table 1**). This implied that glucose was probably involved in the polysaccharide−protein complex and that it was released after protease treatment.

The values of d*n*/d*c* obtained by a differential refractometer were 0.16 and 0.14 mL/g for the alkali extract of the dark husk Job's tears, and 0.15 and 0.14 mL/g for that of the white husk Job's tears before and after protease treatment, respectively. It was noticed that the reduction of protein and the increment of polysaccharide content decreased the value of d*n*/d*c*.

After treating with protease, the [η] of the alkali extracts increased from 0.29 to 0.38 dL/g (the dark husk) and 0.20 to 0.29 dL/g (the white husk) probably due to a higher concentration of polysaccharides. When the alkali extracts were dissolved in deionized water or 0.05 M NaCl, the [η] values were not significantly different. It implied that the polysaccharides had a low degree of charge, which was in agreement with the existence of low uronic acid.

Surface Tension and Rheological Flow Behavior. Compared to the surface tension at the air/water interface (72 dynes/cm), the alkali extracts demonstrated the ability to reduce the surface tension of pure water. It was observed that the surface tension decreased constantly with increasing concentration of the alkali extract in the range of 0.2−1.0% w/v. The reduction of protein level negatively influenced the surface activity of the extracts. For example, the surface tension of a 1.0% (w/v) alkali extract was found to be 57.5 dynes/cm (the dark husk) and 56.5 dynes/cm (the white husk); they were increased to 62.0 and 59.0 dyne/cm, respectively after protease treatment. This may be related to a decrease in protein content and the average MW of arabinoxylans.

Figure 3 illustrates that the alkali extract solution of the dark and white husk Job's tears before and after protease treatment exhibited Newtonian flow behavior, in which the apparent viscosity was independent of shear rate, although the concentration increased from 5 to 20% (w/v). The low viscosity of the alkali extracts might be attributed to the great distribution of substituted arabinose to xylose residues (*33*), and its combination with high protein and mineral contents.

Methylation Analysis of Alkali Extractable Nonstarch Polysaccharides. After methylation, the individual peak of PMAA and fragmentation patterns was identified by their retention time in GC and comparison to mass spectra patterns in the literature (*34*) as well as on the basis that the configurations of arabinose and xylose in the arabinoxylans of cereals were L-furanose and D-pyranose, respectively. The analysis of PMAA and linkage patterns of the alkali extractable nonstarch polysaccharides are shown in **Table 2**.

The results revealed that the alkali extractable nonstarch polysaccharides of the dark and white husk types had similar linkages. The arabinofuranosyl residues (Ara*f*) and xylopyranosyl residues (Xyl*p*) were assigned to arabinoxylans, which were mainly composed of Ara*f* present as nonreducing terminal units (T-Ara*f*) with respect to the high product of 2,3,5-tri-*O*-methyl arabinitol. The T-Ara*f* suggested that a unit of Ara*f* attached to (1,4)-linked xylan as a side chain. The Ara*f* were highly substituted at the xylan backbone through position O-3 of the individual Xyl*p*, observed from the high content of 2-*O*-methyl pentitol and 2,4-di-*O*-methyl pentitol, followed by O-2 and O-3 on the same Xyl*p* as indicated from 1-deuterio pentitol pentaacetate and 4-*O*-methyl pentitol, respectively. The unsubstituted Xyl*p* residues were evidenced from 2,3-di-*O*-methyl pentitol. The O-2 linked Ara*f* was not observed in this study. For both dark and white husk Job's tears arabinoxylans, the terminal Ara*f* were predominantly linked at O-3 of Xyl*p*, similar to the water extractable arabinoxylans reported in wheat flour (*24*) and rye grain (*35*). The Ara*f* to Xyl*p* (Ara*f*/Xyl*p*) ratio of the dark husk type was 0.6, similar to 0.5 of the white husk type. However, it was noticed that the Ara*f*/Xyl*p* ratio was lower than that determined using the HPAEC, possibly because conversion to methylated sugar was not completed or because Ara*f* were lost due to rapid evaporation. The galactopyranosyl residues (Gal*p*) were proposed to be (1,4)- and (1,6)-linked backbones of arabinogalactans (*29*) with Ara*f* attaching at O-2 in the presence of 3,6-di-*O*-methyl hexitol.

^{1}H NMR, ^{13}C NMR, and 2D NMR Analysis. Since there were no major differences between the linkages of the alkali extractable nonstarch polysaccharides from the dark and white husk types, the former was subjected to NMR analysis. The structural features of the arabinoxylans were further elucidated by analysis of NMR spectra. On the basis of the literature for cereal nonstarch polysaccharides, the signals in the region of 5.2−5.4 ppm of ^{1}H NMR analysis were assigned to anomeric protons of α-L-linked-Ara*f*, whereas the signals at 4.4−4.8 ppm were assigned to anomeric protons of *β*-D-linked-Xyl*p* (*27, 31,*

35−*37*). As compared to the chemical shift of known spectra, the region of the observed values at 5.10−5.38 ppm given by the ^{1}H NMR spectra (**Figure 4A**) was assigned to the typical signals of anomeric protons of Ara*f*. The high signal at 5.38 ppm was attributed to the anomeric proton of the α-L-terminal Ara*f* linked to position O-3 of Xyl*p* (**Table 3**). The two signals of similar relative intensity at 5.20 and 5.25 ppm were responsible for the anomeric proton of the α-L-Ara*f* linked to O-2 and O-3 of the double-branched Xyl*p*, respectively. The results suggested that the Xyl*p* were highly substituted at O-3 by α-L-Ara*f*, which was consistent with the methylation data. There was an additional resonance for the anomeric proton at 5.10 ppm, which might be associated with *β*-L-Ara*f* (*38*). However, this regional signal did not appear in the ^{13}C NMR spectra. The unsubstitution of Xyl*p* was reflected from the broad resonance at 4.46 ppm, and the two small peaks at 4.58 and 4.61 ppm were assigned to mono- and disubstituted Xyl*p*, respectively. The spectra did not display resonances in the region of phenolic moieties between 6−8 ppm (*39*) in the ^{1}H NMR, implying no phenolic proportion in the alkali extractable arabinoxylans.

The ^{13}C NMR analysis was used for more accurate identification of the complexity of structural features of the arabinoxylans. The spectra exhibited the well resolved signal characteristics of Ara*f* and Xyl*p* as shown in **Figure 4B**. With respect to anomeric carbons, the α-L-linked Ara*f* observed at 111.3, 110.7, and 110.3 ppm were identified as O-2 and O-3, and O-3 linked to the individual Xyl*p*, compared with those values reported in the literature (*27, 31, 33, 37, 40, 41*). The resonance due to the anomeric carbons of *β*-D-linked Xyl*p* at 103.9, 102.5, and 100.3 ppm were assigned to unsubstituted, monosubstituted at O-3, and disubstituted at O-2 and O-3 Xyl*p*, respectively. The minority of galactose was not assigned in the spectra due to low intensity. Signals of the carboxyl carbons at lower field between 170−180 ppm were not observed, verifying the absence of uronic acid.

The HMQC spectrum (**Figure 5**) showed the spectrum of distinct three cross peaks in the anomeric region of Ara*f*. The C-1 signal at 111.3 and 110.7 ppm, assigned to the α-L-Ara*f* linked to O-2 and O-3 of Xyl*p*, showed the cross peaks with the H-1 resonance at 5.20 and 5.25 ppm, respectively. The C-1 at 110.3 ppm was assigned to the α-L-Ara*f* linked to O-3 of Xyl*p* since it was connected to the H-1 resonance at 5.38 ppm. It was noted that the H-1 at 5.10 ppm assigned to *β*-L-Ara*f* was not correlated with the C-1 region of Ara*f* but the C-1 region of Xyl*p*. The H-1 at 4.46 ppm was assigned to the unsubstituted *β*-D-xylopyranosyl core, showing correlation to C-1 at 103.9 ppm. The cross peak between C-1 and H-1 resonances of mono- and disubstituted Xyl*p* was not observed in this spectrum. The investigation by the HMBC spectrum (**Figure 6**) indicated the cross peaks at the H-1/C-3 of O-3 linked-Ara*f* and the H-1/C-2 of O-3 and O-2 linked-Ara*f*. The COSY assigned the chemical shifts of anomeric protons from the O-2 and O-3 linked-Ara*f* coupling with respective H-2s and the H-1s of mono- and disubstituted Xyl*p* and respective H-2s. Because of limited resolution in the COSY spectrum as a result of very crowded H-3, H-4, and H-5 protons, it was not clear to assign a correlation between these cross peaks (data not shown).

The TOCSY spectrum provided all intra-residue assignments of all protons on each O-3, and O-2 and O-3 linked-Ara*f* as shown in **Figure 7**. Three resonances of mono-, di-, and unsubstituted Xyl*p* were also present in the spectrum. All peak assignments according to ^{1}H and ^{13}C NMR, HMQC, HMBC, COSY, and TOCSY, along with the literature are summarized in **Table 3**.

In summary, the predominant alkali extractable nonstarch polysaccharides from both Job's tears were arabinoxylans with a high Ara/Xyl ratio of 1.21−1.25, indicating a highly branched structure. The average MW of arabinoxylans reduced after treatment with protease, which probably could represent covalent cross-linkages between arabinoxylans and protein. The Newtonian flow behavior at high concentration (5−20% w/v) of the alkali extracts could be attributed to the highly substituted arabinoxylans with high protein and mineral contents. There were no significant differences in linkage structure between the two types. The terminal α-L-Ara*f* were predominantly linked to O-3 of (1,4)-linked *β*-D-Xyl*p* on the backbone and some were also attached to both O-2 and O-3 positions.

ABBREVIATIONS USED

Ara/Xyl, arabinose to xylose; MW, molecular weight; Ara*f* arabinofuranosyl residues; Xyl*p* xylopyranosyl residues; Gal*p* Galactopyranosyl residues; HPAEC, high performance anion exchange chromatography; HPSEC, high performance size exclusion chromatography; d*n*/d*c*, refractive index increment; Da, dalton; RI, refractive index; LS, light scattering; UV, ultraviolet; PMAA, partially methylated alditol acetates; ^{1}H NMR, proton nuclear magnetic resonance; ^{13}C NMR, ^{13}C nuclear magnetic resonance; HMQC, heteronuclear multiple quantum coherence; HMBC, long-range heteronuclear correlation; COSY, proton/proton correlation; TOCSY, total proton/proton correlation.

ACKNOWLEDGMENT

We are thankful to Cathy Wang and Ben Huang for technical assistance and John Nikiforuk and Dr. Barbara Blackwell of Eastern Cereal and Oilseed Research Centre, AAFC, for providing the NMR analysis.

LITERATURE CITED

(1) Chang, H.-C.; Huang, Y.-C.; Hung, W.-C. Antiproliferative and chemopreventive effects of adlay seed on lung cancer in vitro and in vivo. *J. Agric. Food Chem.* **2003**, *51*, 3656–3660.

(2) Hung, W.-C.; Chang, H.-C. Methanolic extract of adlay seed suppresses COX-2 expression of human lung cancer cells via inhibition of gene transcription. *J. Agric. Food Chem.* **2003**, *51*, 7333–7337.

(3) Hsu, H.-Y.; Lin, B.-F.; Lin, J.-Y.; Kuo, C.-C.; Chiang, W. Suppression of Allergic reactions by dehulled adlay in association with the balance of Th1/Th2 cell responses. *J. Agric. Food Chem.* **2003**, *51*, 3763–3769.

(4) Kuo, C.-C.; Chiang, W.; Liu, G.-P.; Chien, Y.-L.; Chang, J.-Y.; Lee, C.-K.; Lo, J.-M.; Huang, S.-L.; Shih, M.-C.; Kuo, Y.-H. 2,2′-Diphenyl-1-picrylhydrazyl radical-scavenging active components from adlay *Coix lachryma-jobi* L.Var. *ma-yuen* Stapf) Hulls. *J. Agric. Food Chem.* **2002**, *50*, 5850–5855.

(5) Check, J. B.; K'Ombut, F. O. The effect on fibrinolytic system of blood plasma of wistar rats after feeding them with Coix mixed diet. *East Afr. Med. J.* **1995**, *72*, 51–55.

(6) Numata, M.; Yamamoto, A.; Moribayashi, A.; Yamada, H. Antitumor components isolated from the Chinese herbal medicine *Coix lachryma-jobi*. *Planta Med.* **1994**, *60*, 356–359.

(7) Gibbs, W. W. Healing cancer. *Sci. Am.* **1998**, *279*, 40–41.

(8) Tsai, C. E.; Yang, L.; Su, P.; Chen, R. M.-Y. Beneficial effect of adlay on plasma lipids in hamsters and hyperlipidemic and diabetic patients. *Atherosclerosis.* **1998**, *136*, S43.

(9) Yu, Y.-M.; Chang, W.-C.; Liu, C.-S.; Tsai, C.-M. Effect of young barley leaf extract and adlay on plasma lipids and LDL oxidation in hyperlipidemic smokers. *Biol. Pharm. Bull.* **2004**, *27*, 802–805.
(10) Yu, Y.-T.; Lu, T.-J.; Chiang, M.-T.; Chiang, W. Physicochemical properties of water-soluble polysaccharide enriched fractions of adlay and their hypolipidemic effect in hamsters. *J. Food Drug Anal.* 2005*13*, 361–367.
(11) Kim, S. O.; Yun, S.-J.; Jung, B.; Lee, E. H.; Hahm, D.-H.; Shim, I.; Lee, H.-J. Hypolipidemic effects of crude extract of adlay seed *Coix lachrymajobi* var. mayuen) in obesity rat fed high fat diet: Relations of TNF-α and leptin mRNA expressions and serum lipid levels. *Life Sci.* **2004**, *75*, 1391–1404.
(12) Wood, P. J. Relationships between solution properties of cereal β-glucans and physiological effects: a review. *Trends Food Sci. Technol.* **2002**, *13*, 313–320.
(13) Lopez, H. W.; Levrat, M.-A.; Guy, C.; Messager, A.; Demigné, C.; Rémésy, C. Effects of soluble corn bran arabinoxylans on cecal digestion, lipid metabolism, and mineral balance (Ca, Mg) in rats. *J. Nutr. Biochem.* **1999**, *10*, 500–509.
(14) AOAC. *Official Methods of Analysis*, 17th ed.; Association of Official Analytical Chemists: Washington DC, 2000.
(15) Wood, P. J.; Weisz, J.; Blackwell, B. A. Molecular characterization of cereal β-D-glucans, structural analysis of oat glucan and rapid structural evaluation of β-D-glucans from different sources by HPLC of oligosaccharides released by lichenase. *Cereal Chem.* **1991**, *68*, 31–39.
(16) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356.
(17) Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484–489.
(18) Huggins, M. L. The viscosity of dilute solutions of linear chain molecules IV. Dependence on concentration. *J. Am. Chem. Soc.* **1942**, *64*, 2716–2722.
(19) Kraemer, E. O. Molecular weights of celluloses and cellulose derivatives. *Ind. Eng. Chem.* **1938**, *30*, 1200–1203.
(20) Ciucanu, I.; Kerek, F. A simple and rapid method for the permethylation of carbohydrates. *Carbohydr. Polym.* **1984**, *131*, 209–217.
(21) Silva, L. P.; Ciocca, M. L. S. Total, insoluble and soluble dietary fiber values measured by enzymatic-gravimetric method in cereal grains. *J. Food Comp. Anal.* **2005**, *18*, 113–120.
(22) Yamada, H.; Yanahira, S.; Kiyohara, H.; Cyong, J-C.; Otsuka, Y. Water- soluble glucans from *Coix lacryma-jobi* var *ma-yuen*. *Phytochemistry* **1986**, *25*, 129–132.
(23) Yamada, H.; Yanahira, S.; Kiyohara, H.; Cyong, J-C.; Otsuka, Y. Characterization of anti-complementary acidic heteroglycans from the seed of *Coix lacryma-jobi* var. *ma-yuen*. *Phytochemistry* **1987**, *26*, 3269–3275.
(24) Scalbert, A.; Monties, B.; Lallemand, J. Y.; Guittet, E. R.; Rolando, C. Ether linkage between phenolic acids and lignin fractions of wheat straw. *Phytochemistry* **1985**, *24*, 1359–1362.
(25) Lott, J. N. A.; Ockenden, I. The Fine Structure of Phytate-Rich Particles in Plants. In *Phytic Acid: Chemistry and Applications*; Graf, E., Ed.; Pilatus Press.: Minneapolis, MN, 1986; pp 43−55.
(26) Gruppen, H.; Hamer, R. J.; Voragen, A. G. J. Water-unextractable cell wall material from wheat flour. 1. Extraction of polymers with alkali. *J. Cereal Sci.* **1992**, *16*, 41–51.
(27) Annison, G.; Choct, M.; Cheetham, N. W. Analysis of wheat arabinoxylans from a large-scale isolation. *Carbohydr. Polym.* **1992**, *19*, 151–159.
(28) Izydorczyk, M. S.; Macri, L. J.; MacGregor, A. W. Structure and physicochemical properties of barley non-starch polysaccharides-II. Alkali- extractable β-glucans and arabinoxylans. *Carbohydr. Polym.* 199835, 259–269.
(29) Vinkx, C; J, A.; Stevens, I.; Gruppen, H.; Grobet, P. J.; Delcour, J. A. Physicochemical and functional properties of rye nonstarch polysaccharides. VI. Variability in the structure of water-unextractable arabinoxylans. *Cereal Chem.* **1995**, *72*, 411–418.
(30) Fincher, G. B.; Stone, B. A. Cell Walls and Their Components in Cereal Grain Technology. In *Advances in Cereal Science and Technology*; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1986; Vol. 8, pp 207−295.
(31) Ebringerová, A.; Hromádková, Z.; Berth, G. Structural and molecular properties of a water-soluble arabinoxylan-protein complex isolated from rye bran. *Carbohydr. Res.* **1994**, *264*, 97–109.
(32) Storsley, J. M.; Izydorczyk, M. S.; You, S.; Biliaderis, C. G.; Rossnagel, B. Structure and physicochemical properties of β-glucans and arabinoxylans isolated from hull-less barley. *Food Hydrocolloid.* **2003**, *17*, 831–844.
(33) Bengtsson, S.; Andersson, R.; Westerlund, E.; Åman, P. Content, structure and viscosity of soluble arabinoxylans in rye grain from several countries. *J. Sci. Food Agric.* **1992**, *58*, 331–337.
(34) Carpita, N. C.; Shea, E. M. Linkage Structure of Carbohydrates by Gaschromatography-Mass Spectrometry (GC-MS) of Partially Methylated Alditol Acetates. In *Analysis of Carbohydrates by GLC and MS*; Bierman, C. J., McGinnis, G. D., Eds.; CRC Press: Boca Raton, FL, 1989; pp 157−216.
(35) Bengtsson, S.; Åman, P. Isolation and chemical characterization of water-soluble ambinoxylans in rye grain. *Carbohydr. Polym.* **1990**, *12*, 267–277.
(36) Westerlund, E.; Andersson, R.; Åman, P. Isolation and chemical characterization of water-soluble mixed-linked β-glucans and arabinoxylans in oat milling fractions. *Carbohydr. Polym.* **1993**, *20*, 115–123.
(37) Hoffmann, R. A.; Kamerling, J. P.; Vliegenthart, J. F. G. Structural features of a water-soluble arabinoxylan from the endosperm of wheat. *Carbohydr. Res.* **1992**, *226*, 303–311.
(38) Ebringerová, A.; Hromádková, Z.; Petráková, E.; Hricovíni, M. Structural features of a water-soluble L-arabino-D-xylan from rye bran. *Carbohydr. Res.* **1990**, *198*, 57–66.
(39) Saulnier, L.; Crépeau, M-J.; Lahaye, M.; Thibault, J-F.; Gracia-Conesa, M. T.; Kroon, P. A.; Williamson, G. Isolation and structural determination of two 5,5′-diferuloyl oligosaccharides indicate that maize heteroxylans are covalently cross-linked by oxidatively coupled ferulates. *Carbohydr. Res.* **1999**, *320*, 82–92.
(40) Hoffmann, R. A.; Roza, M.; Maat, M.; Kamerling, J. P.; Vliegenthart, J. F. G. Structural Characteristics of the warm-water-soluble arabinoxylans from the tailings of the soft wheat variety Kadet. *Carbohydr. Polym.* **1991**, *16*, 275–289.
(41) Izydorczyk, M. S.; Biliaderis, C. G. Structural heterogeneity of wheat endosperm arabinoxylans. *Cereal Chem.* **1993**, *70*, 641–646.

Received for review April 18, 2008. Revised manuscript received July 12, 2008. Accepted July 14, 2008. This work was supported by Suranaree University of Technology, Suratthani Rajabhat University, Thailand, and Agriculture and Agri-Food Canada, Canada. Most of the research was carried out in Guelph Food Research Centre, Agriculture and Agri-Food Canada.

JF801231Y