# Removal of Arabinose Substituents from Corn Pericarp Arabinoxylan

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## Abstract

Partial acid hydrolysis of corn pericarp arabinoxylan (arabinose/xylose (A/X) ratio 0.35 and mean molecular weight of 53.6 × $10^3$) was carried out at pH 1.0 for 1 ~ 6 h at 37 ~ 57 °C to remove arabinose substituents. The removal of arabinose could be described by regression analysis with the method of least squares. Following the regression profile, three kinds of desubstituted arabinoxylans having A/X ratios of 0.25, 0.12 and 0.03 with mean molecular weight values of 37.3 × $10^3$, 15.6 × $10^3$ and 7.2 × $10^3$, respectively, could be prepared. All corn pericarp arabinoxylans were in the amorphous state and the film formability of the native state was lost after the partial acid hydrolysis.

**Keywords:** removal of arabinose, partial acid hydrolysis, corn pericarp arabinoxylan, arabinose substituents.

## Introduction

Corn pericarp, rich in hemicellulose, is a waste of industrial corn starch production. Its global production in the marketing year of October 2010 ~ September 2011 was estimated to be over 4 million tons, calculated from the percentage of the world corn production (831 million tons, USDA FAS Grain: World Markets and Trade Report FG 12-12) used for food manufacture (8.6%, Kim and Dale 2004) multiplied by the percentage pericarp content (6.2%, Wolf *et al.* 1969). Although it is used as animal feed with the addition of corn protein (Shukla and Cheryan 2001) and its use as an emulsifier has been investigated (Yadav *et al*. 2007 and 2008), development of more valuable applications is desirable. Previously we optimized solubilization of carbohydrates from corn pericarp by microwave irradiation (Yoshida *et al.* 2010) and demonstrated the effectiveness of a NaOH-urea solvent system for extraction of arabinoxylan mixed with β-glucan from corn pericarp, to form flexible transparent films (Yoshida *et al*. 2012).

Arabinoxylan, a principal hemicellulose of corn pericarp, has a backbone of β-(1,4)-linked D-xylose substituted with side chains consisting largely of α-L-arabinose on *O*-3 and/or *O*-2 of the xylose residue (Saulnier *et al*. 1995). Recently, xylose-based oligosaccharides have been reported to possess various bioactivities, such as lowering blood cholesterol level, reduction of stomach ulcer lesions, improvement of mineral absorption, regulation of lipid metabolism, decrease in the risk of colon cancer and modulation of the immune system (Swennen *et al*. 2006). These valuable properties make corn arabinoxylan attractive as an ingredient in functional foods (Crittenden and Playne 1996). Previous studies indicate that the profile of arabinose substitution of arabinoxylan greatly alters its functional properties, such as solubility, water holding capacity and viscoelastic properties (Izydorczyk and Biliaderis 1992 and 1995). The degree of arabinose substitution in arabinoxylan significantly varies according to species of crops, parts of plant and growth conditions such as seasons, types of soil, weather, etc. (Izydorczyk and Biliaderis 1995), making artificial regulation of its substitution necessary for characterization of the role of arabinose in the properties of corn pericarp arabinoxylan. So far, two kinds of regulation method have been tried, one is enzymatic treatment (Höije *et al*. 2008) and the other is partial acid hydrolysis (Sternemalm *et al*. 2008). By using arabinofuranosidase, highly controlled removal of arabinose residues was possible without reduction of the β-(1,4) linked xylan chain length (Höije *et al*. 2008). However, the commercial availability of the specific enzymes is an obstacle for this method. In this study, therefore, the partial acid hydrolysis method was used to remove the arabinose substituents from corn pericarp arabinoxylan. Regression analysis with the method of least squares has been applied to describe the removal of arabinose from the corn pericarp arabinoxylan.

## Materials and Methods

Dried corn pericarp supplied from a corn starch manufacturer (Sanwa Cornstarch Co., Ltd.) in Nara Prefecture, Japan, was treated twice with hot water (121 °C, 1 h) and dried overnight at 45 °C. Arabinoxylan was prepared from corn pericarp as described previously (Yoshida *et al*. 2012) with a slight modification. Corn pericarp was treated three times with dimethyl sulfoxide at room temperature to remove starch, and the residue was extracted three times with 4% KOH at room temperature. The extracted solution was treated by graduated ethanol precipitation. The materials precipitated between 30 ~ 50% EtOH were obtained as arabinoxylan. The isolated arabinoxylan was solubilized in distilled water at 70 °C to make 1% concentration. For partial acid hydrolysis this solution was adjusted to pH 1.0 with hydrochloric acid and incubated for 1 ~ 6 h at 37 ~ 57 °C. Ethanol pre-cooled to 4 °C was added to make about 70% ethanol and the precipitated materials were recovered by centrifugation. Precipitants were washed with 70% ethanol, ethanol, acetone, and dried at 60 °C to give partially degraded corn pericarp arabinoxylan samples.

The monosaccharide composition of the arabinoxylan samples was analyzed by high-performance anion exchange chromatography (HPAEC) on a Dionex DX-500 system (Sunnyvale, CA, USA) with a pulsed amperometry detector (ED-40) after complete hydrolysis according to the Saeman method (1945). The molecular weight distribution of the arabinoxylan samples was analyzed with size exclusion chromatography on a column of YMC-Pack Diol-120 at 25 °C using pullulans (Shodex Standard P-82, Showa Denko, Co., Ltd.) as calibration standards. The eluent was 5 mM sodium phosphate buffer containing 0.1 M NaCl, pH 6.8, and the flow rate was 0.6 mL/min. Elution was monitored using a refractive index detector (TOSO, RI-8) and recorded by a Waters 741 Data Module. X-Ray diffraction profiles of the arabinoxylan samples were measured by Rigaku Ultima IV, using Ni filtered Cu-Kα radiation (λ = 0.1542 nm) at 40 kV and 40 mA. The scanning of 2 $\theta$ (5 ~ 40°) was conducted with speed of 2°/min. JMP 9 (SAS Institute, Inc.) was used for data analysis.

## Results and Discussion

The relative monosaccharide compositions of the untreated arabinoxylan and the partially acid-hydrolyzed arabinoxylans are listed in Table 1. The raw corn pericarp arabinoxylan has a relative carbohydrate composition of 66.3% xylose, 23.3% arabinose and 8.7% galactose. In general, the degree of arabinose substitution in arabinoxylan is denoted by the molar ratio of arabinose to xylose (A/X). The value of A/X (0.35) for the corn pericarp was slightly lower than those of arabinoxylans from various cereal grains so far reported, 0.50 ~ 0.65 (corn), 0.50 ~ 1.07 (wheat) and 0.48 ~ 0.78 (rye) (Saulnier *et al.* 1995; Yadav *et al.* 2007; Izydorczyk and Biliaderis 1995). During partial acid hydrolysis, the A/X value decreased with increasing hydrolysis temperature and time, reaching 0.02 at 57 °C for 6 h (Table 1), indicating effective removal of arabinose residues in accord with the observation reported by Whistler and Corbett (1955) and Whistler and BeMiller (1956).

The effects of the hydrolysis temperature and time on the extent of removal of arabinose from the corn pericarp arabinoxylan were further evaluated by regression analysis with the method of least squares (Figure 1). When the value of A/X was used as the degree of removal of arabinose, the regression model was the following equation 1:

$$\begin{aligned} Y \;&= 7.087 \times 10^{-1} - 5.357 \times 10^{-2} X_1 - 1.421 \times 10^{-2} X_2 \\ &\quad - 7.533 \times 10^{-4} X_1X_2 + 8.373 \times 10^{-3} X_1X_1 \\ &\quad + 1.189 \times 10^{-4} X_2X_2 \quad \text{......} \quad (1) \end{aligned}$$

where, $Y$ is the value of the A/X ratio of arabinoxylan after partial acid hydrolysis, and $X_1$ and $X_2$ are the hydrolysis time (h) and temperature (°C), respectively. After the regression model was analyzed using analysis of variance, the correlation coefficient of the regression model was as high as 0.98, and its $p$-value was significant at the probability level of 5% (Table 2). The present regression model revealed that the effect of hydrolysis temperature on the release of arabinose by dilute acid hydrolysis was stronger than that of treatment time, in good agreement with the previous observation of Sternemalm *et al*. (2008) for rye arabinoxylan. The regression analysis also allowed the prediction of hydrolysis conditions to yield various A/X ratios from corn pericarp arabinoxylan. Thus, the partial acid hydrolysis conditions for the preparation of corn pericarp arabinoxylans with A/X values of 0.25, 0.12 and 0.03 are expected to be 37 °C for 1 h, 47 °C for 3 h and 57 °C for 3 h, respectively, according to the regression model (Figure 1).

The degree of substitution by arabinose in arabinoxylan remarkably affects its solubility due to intermolecular hydrogen bonds between unsubstituted xylan chains (Nieduszynski and Marchessault 1972; Andrewartha *et al*. 1979) disrupting hydration. Therefore, the solubility of the three kinds of corn pericarp arabinoxylans in water was analyzed (Table 3). The results showed that fairly good solubility was still maintained or the arabinoxylans with low A/X ratios, but a clear trend towards decreasing solubility with lowering the A/X ratios is evident.

Table 1. Relative monosaccharide composition of the untreated arabinoxylan and partially hydrolyzed arabinoxylans

| Hydrolysis condition | | Relative monosaccharide composition (%, w/w) | | | | | Ara/Xyl |
|---|---|---|---|---|---|---|---|
| Temperature (°C) | Time (h) | Ara | Gal | Glc | Xyl | Man | |
| Untreated arabinoxylan | | 23.3 | 8.7 | 1.7 | 66.3 | tr | 0.35 |
| | | *Partial acid hydrolysis* | | | | | |
| 37 | 1 | 18.0 | 8.4 | 5.2 | 68.2 | 0.1 | 0.26 |
| 37 | 3 | 12.6 | 6.5 | 13.7 | 67.2 | tr | 0.19 |
| 37 | 6 | 11.7 | 7.5 | 6.2 | 74.4 | 0.2 | 0.16 |
| 47 | 1 | 14.7 | 8.7 | 4.2 | 66.4 | 6.0 | 0.22 |
| 47 | 3 | 9.4 | 6.6 | 4.8 | 76.8 | 2.4 | 0.12 |
| 47 | 6 | 4.8 | 5.0 | 6.7 | 77.8 | 5.7 | 0.06 |
| 57 | 1 | 14.0 | 8.6 | 4.4 | 67.5 | 5.6 | 0.21 |
| 57 | 3 | 4.5 | 3.5 | 3.7 | 88.3 | tr | 0.05 |
| 57 | 6 | 1.4 | 2.7 | 10.0 | 75.9 | 10.0 | 0.02 |

tr: trace

Table 2. Analysis of variance for the regression model

| | Degree of freedom | Sum of square | Mean square | *F*-value | *p*-value |
|---|---|---|---|---|---|
| Model | 5 | 0.0572 | 0.0114 | 34.4482 | 0.0072 |
| Residual | 3 | 0.0010 | 0.0003 | | |
| Total | 8 | | | | |
| $R^2$ | 0.98 | | | | |

Table 3. Effects of the acid hydrolysis of arabinoxylan on solubility in water

| Hydrolysis condition | | Solubility (%) | A/X |
|---|---|---|---|
| Temperature (°C) | Time (h) | | |
| Untreated arabinoxylan | | 99.1 | 0.35 |
| | *Partial acid hydrolysis* | | |
| 37 | 1 | 93.1 | 0.26 |
| 47 | 3 | 88.0 | 0.12 |
| 57 | 3 | 86.2 | 0.05 |

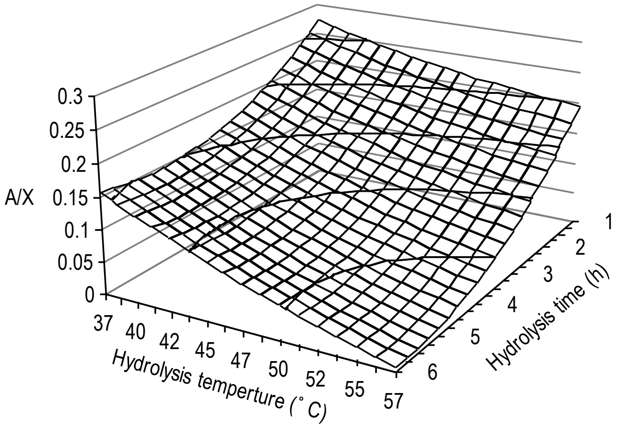

Figure 1. Effects of hydrolysis temperature and time on removal of arabinose from corn pericarp arabinoxylan.

The molecular weight distributions of the untreated arabinoxylan and partially hydrolyzed arabinoxylans at pH 1.0 were investigated using SEC by dissolving in 5 mM sodium phosphate buffer containing 0.1 M NaCl (pH 6.8) (Figure 2). The untreated arabinoxylan showed a broad elution profile having a peak around 28 min which was equivalent to a mean molecular weight (*Mw*) of 53.6 × $10^3$. This value is within the range given for arabinoxylans from corn fiber (9.3 × $10^3$ to 300 × $10^3$, Yadav *et al.* 2008). After partial acid hydrolysis at pH 1.0, however, the *Mw* values of the recovered arabinoxylans decreased to approximately 37.3 × $10^3$, 15.6 × $10^3$ and 7.2 × $10^3$ for the A/X values of 0.25, 0.12 and 0.03, respectively. In the case of the partial hydrolysis of the rye arabinoxylan with 0.025 M oxalic acid at 60 °C, the A/X and *Mw* values went from 0.52 and 305 × $10^3$ before hydrolysis to 0.36 and 8.6 × $10^3$, respectively (Sternemalm *et al.* 2008). Further hydrolysis produced aggregates over an A/X range of 0.31 ~ 0.23, and precipitation took place below A/X values of 0.1. Similar agglomeration was also observed in enzymatically treated rye arabinoxylans with A/X ratios of 0.30 and below (Höije *et al.* 2008). In contrast, no such remarkable behavior was detected for the corn pericarp arabinoxylan (Table 3). This variation may be caused by differences in the molecular weight and substitution profile of the two arabinoxylans. In addition, the present partial acid hydrolysis of the corn pericarp arabinoxylan indicates that hydrolysis at lower temperature for longer time resulted in removal of arabinose substituents with less degradation of the main xylan chain.

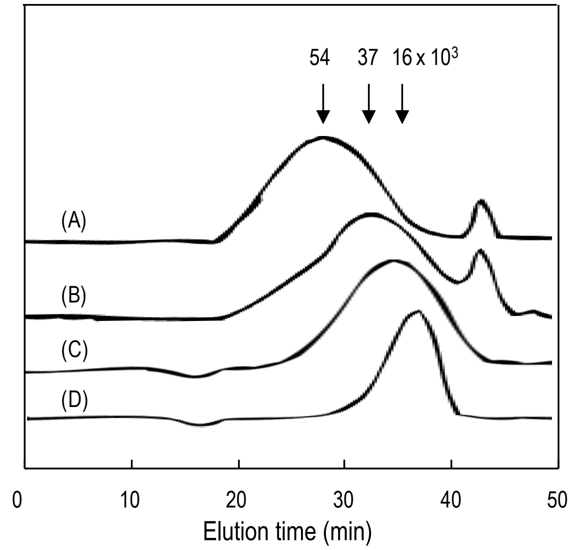

Figure 2. Molecular weight distribution of corn pericarp arabinoxylan samples; untreated arabinoxylan (A) and arabinoxylan hydrolyzed at pH 1.0 and 37 °C for 1 h (B), at 47 °C for 3 h (C) and at 57 °C for 3 h (D). The A/X ratios of arabinoxylan samples are 0.35 (A), 0.25 (B), 0.12 (C) and 0.03 (D).

Next, the crystalline properties of the corn pericarp arabinoxylans were analyzed by X-ray diffraction (Figure 3). All X-ray diffraction patterns showed a broad peak centered at around 18°, indicating the presence in amorphous state. Dea and Rees (1973) reported that lower branched arabinoxylan from sugar cane was recovered as crystalline aggregates. Nieduszynski and Marchessault (1972) reported that pure (1,4)-β-D-xylan was able to crystallize if dried slowly. Höije *et al*. (2008) prepared rye arabinoxylans with A/X ratios between 0.50 ~ 0.20 by enzyme treatment and showed the semi-crystalline properties of arabinoxylans which had A/X ratios below 0.30. They also reported that all samples formed cohesive films upon drying without addition of external plasticizers. With regard to the film formation of the corn pericarp arabinoxylans, only the untreated one could form a film after simple drying of its aqueous solution (Yoshida *et al*. 2012). All the samples of desubstituted corn pericarp arabinoxylans had lost the ability to form films probably due to their low molecular weights.

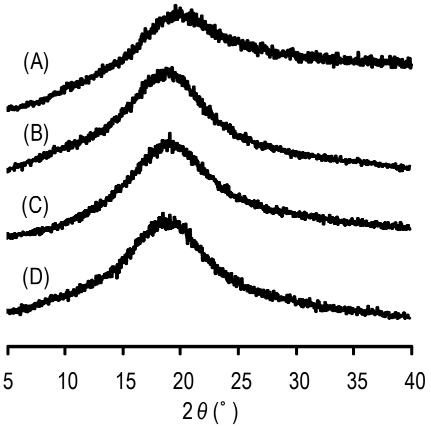

Figure 3. X-Ray diffraction patterns of arabinoxylan samples; untreated arabinoxylan (A) and arabinoxylan hydrolyzed at 37 °C for 1 h (B), at 47 °C for 3 h (C) and at 57 °C for 3 h (D). The A/X ratios of arabinoxylan samples are 0.35 (A), 0.25 (B), 0.12 (C) and 0.03 (D).

## Conclusions

Removal of arabinose substituents from corn pericarp arabinoxylan (A/X ratio 0.35 and mean molecular weight of 53.6 × $10^3$) was carried out by partial acid hydrolysis at pH 1.0 for 1 ~ 6 h at 37 ~ 57 °C. The extent of removal was followed by regression analysis with the method of least squares. Using the regression profile, three kinds of desubstituted arabinoxylans having A/X ratios of 0.25, 0.12 and 0.03 with mean molecular weights of 37.3 × $10^3$, 15.6 × $10^3$ and 7.2 × $10^3$ could be prepared. All corn pericarp arabinoxylans were in the amorphous state and the film forming ability of the native state was lost after partial acid hydrolysis.

## Acknowledgements

This study was supported in part by a Grant in Aid from the Ministry of Education, Culture, Sports, Science and Technology Japan.

## References

Andrewartha, K.A.; Phillips, D.R.; Stone, B.A. 1979. Solution properties of wheat-flour arabinoxylans and enzymically modified arabinoxylans. Carbohydr Res. 77: 191-204.

Crittenden, R.G.; Playne, M.J. 1996. Production, properties and applications of food-grade oligosaccharides. Trends Food Sci Technol. 7: 353-361.

Dea, I.C.M.; Rees, D.A.; Beveridge, R.J.; Richards, G.N. 1973. Aggregation with change of conformation in solutions of hemicellulose xylans. Carbohydr Res. 29: 363-372.

Höije, A.; Sternemalm, E.; Heikkinen, S.; Tenkanen, M.; Gatenholm, P. 2008. Material properties of films from enzymatically tailored arabinoxylans. Biomacromolecules. 9: 2042-2047.

Izydorczyk, M.S.; Biliaderis, C.G. 1992. Effect of molecular size on physical properties of wheat arabinoxylan. J Agric Food Chem. 40: 561-568.

Izydorczyk, M.S.; Biliaderis, C.G. 1995. Cereal arabinoxylans: advances in structure and physicochemical properties. Carbohydr Polym. 28: 33-48.

Kim, S.; Dale, B.E. 2004. Global potential bioethanol production from wasted crops and crop residues. Biomass Bioenergy. 26: 361-375.

Nieduszynski, I.A.; and Marchessault, R.H. 1972. Structure of β,D(1→4')-xylan hydrate. Biopolymers. 11: 1335-1344.

Saeman, J.F.; Bubl, J.L.; Harris, E.E. 1945.Quantitative saccharification of wood and cellulose. Ind Eng Chem Anal Ed. 17: 35-37.

Saulnier, L.; Marot, C.; Chanliaud, E.; Thibault, J.-F. 1995. Cell wall polysaccharide interactions in maize bran. Carbohydr Polym. 26: 279-287.

Shukla, R.; Cheryan, M. 2001. Zein: the industrial protein from corn. Ind Crops Prod. 13: 171-192.

Sternemalm, E.; Höije, A.; Gatenholm, P. 2008. Effect of arabinose substitution on the material properties of arabinoxylan films. Carbohydr Res. 343: 753-757.

Swennen, K.; Courtin, C.M.; Delcour, J.A. 2006. Non-digestible oligosaccharides with prebiotic properties. Crit Rev Food Sci Nutr. 46: 459-471.

USDA FAS Grain: World Markets and Trade Report, Circular Series FG 12-12, December 2012 (www.fas.usda.gov/psdonline/circulars/grain.pdf).

Whistler, R.L.; Corbett, W.M. 1955. Oligosaccharides from partial acid hydrolysis of corn fiber hemicellulose. J Am Chem Soc. 77; 6328-6330.

Whistler, R.L.; BeMiller, J.N. 1956. Hydrolysis components from methylated corn fiber gum. J Am Chem Soc. 78: 1163-1165.

Wolf, M.J.: Cull, I.M.; Helm, J.L.; Zuber, M.S. 1969. Measuring thickness of excised mature corn pericarp. Agron J. 61: 777-779.

Yadav, M.P.; Johnston, D.B.; Hicks, K.B. 2007. Structural characterization of corn fiber gums from coarse and fine fiber and a study of their emulsifying properties. J Agric Food Chem. 55: 6366-6371.

Yadav, M.P.; Parris, N.; Johnston, D.B.; Hicks, K.B. 2008. Fractionation, characterization, and study of the emulsifying properties of corn fiber gum. J Agric Food Chem. 56: 4181-4187.

Yoshida, T.; Tsubaki, S.; Teramoto, Y.; Azuma, J. 2010. Optimization of microwave-assisted extraction of carbohydrates from industrial waste of corn starch production using response surface methodology. Bioresour Technol. 101: 7820-7826.

Yoshida, T.; Sakamoto, M.; Azuma, J. 2012. Extraction of hemicelluloses from corn pericarp by the NaOH-urea solvent system. Procedia Chem. 4: 294-300.

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