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Extraction of Hemicelluloses from Corn Pericarp by the NaOH-Urea Solvent System

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Abstract

The NaOH-urea solvent system was applied for solubilization of hemicelluloses in corn pericarp (CP), an industrial waste of corn starch production, and the mechanical properties of films prepared from the isolated hemicelluloses were analyzed. CP was soaked in 0-8 wt% NaOH solutions containing 0-8 M urea, and the mixtures were frozen at -20 °C and thawed. By a simple recovery of the thawed solutions by filtration hemicelluloses were found to be solubilized efficiently above 2 wt% NaOH. The results of sugar compositional analysis indicate that the extracted materials were mixtures of hemicelluloses composed of arabinoxylan and β -(1,3;1,4)-glucan having arabinose/xylose ratios of 0.84-0.72. The present results indicated that NaOH solutions containing urea, with concentrations not enough to solubilize cellulose were adequate for extraction of hemicelluloses in CP. The hemicellulose extracted with 2 wt% NaOH-6 M urea could form transparent films whose mechanical properties were 56.2 MPa, 3.5% and 3.09 GPa for breaking stress (σ_{\max}), maximum strain (ϵ_{\max}) and elastic modulus (E), respectively, as evaluated by tensile tests. These values were 1.2-, 1.3- and 0.94-fold higher than those obtained by the film of arabinoxylan alone. Results suggest that β -glucan gives mechanical strength and flexibility to the stiff arabinoxylan films.

© 2012 Published by Elsevier Ltd. Open access under [CC BY-NC-ND license](http://creativecommons.org/licenses/by-nc-nd/3.0/).*Keywords:* Corn pericarp; hemicelluloses; arabinoxylan; solubilization; NaOH-Urea.

1. Introduction

Corn pericarp (CP), a waste of industrial corn starch production, is rich in hemicelluloses. Therefore, characterization of its hemicelluloses is the primal target for its practical utilization. Major hemicellulose in CP is arabinoxylan consisting of a backbone of β -(1,4)-linked D-xylose with side chains of α -L-arabinose [1]. Recently, corn arabinoxylan was suggested for use as a food additive because of its benefit

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for human health and good emulsion property [2]. Hemicelluloses in CP are isolated by extraction with alkali and acid solutions [3,4], steam fractionation [5], hot compressed water extraction [6] and microwave-assisted heating [7].

The NaOH-urea solvent system has been developed for solubilization of cellulose [8]. This cheap and environmentally friendly aqueous solvent can rapidly dissolve cellulose at low temperature. However, no study has investigated its application in solubilizing hemicellulosic polysaccharides.

The present study was intended to clarify the feasibility of this solvent system for solubilization of constituents in CP. Results showed that this solvent system is effective for the selective solubilization of hemicelluloses in CP. Mechanical properties of films prepared from isolated hemicelluloses were also compared with those of arabinoxylan and β -glucan films and the results suggest that β -glucan gives mechanical strength and plasticity to the arabinoxylan films.

2. Experiment

2.1. Materials

Kernels of sweet corn cultivated in Hokkaido, Japan, were purchased from Kewpie Co., Japan. Beta-glucan from oat and β -glucan enzymatic assay kit were purchased from Sigma (St Louis, Missouri, U.S.A.) and Megazyme (Megazyme International Ireland Ltd., Wicklow, Ireland), respectively.

2.2. Methods

Preparation of corn pericarp and arabinoxylan. Corn kernels were vertically cut into two parts and CP was manually taken out from each upper half portion of the excised pericarps. CP was treated with hot water (121 °C, 1 h), and dried over night at 45 °C. Arabinoxylan was prepared from CP with the modified method of Dervilly *et al.* [9]. CP was treated with dimethylsulfoxide twice at room temperature, and the residue was extracted with 4% KOH at room temperature. Extracted materials were recovered by graduated ethanol precipitation. The materials precipitated between 30-50% of EtOH were obtained as arabinoxylan.

Compositional analysis. Protein content was determined from elemental analysis by using 6.25 as a protein conversion factor according to the AOAC official method 32.2.02. Lipid content was determined from chloroform-methanol extraction. Ash, β -glucan and starch contents were determined according to the AOAC official methods of 32.1.05., 995. 16. and 996. 11., respectively. Cellulose, hemicellulose and pectin contents were determined according to the method of López-Casado *et al.* [10]. Uronic acid content was determined according to the method of Filisetti-Cozzi and Carpita [11]. Monosaccharide composition of CP was analyzed by high-performance anion exchange chromatography (HPAEC) on a column of CarboPac PA1 (4 × 250 mm, Dionex, Sunnyvale, CA, USA) with pulsed amperometric detector (ED-40) using 1.0 mM NaOH as a mobile phase after hydrolysis of the sample according to the method of Seaman *et al.* [12].

Extraction of corn pericarp with NaOH-urea solution. CP was soaked in 0-8 wt% NaOH solution containing 6 M urea under the fixed liquid to solid ratio of 125 mL/g and kept at -20 °C. After thawing, the solutions were filtrated to separate the solubilized fraction from residues. The solubilized fraction was neutralized with acetic acid, dialyzed against water and subjected to EtOH precipitation. The precipitated materials were recovered by filtration, and dried over night at 45 °C. Effects of the NaOH-urea treatment

on morphological properties of CP were investigated by low voltage scanning electron microscope (LV-SEM, VE-8800, Keyence Co., Osaka, Japan) at 1.7 kV on an amorphous carbon stage.

Preparation and mechanical analysis of films. The hemicelluloses extracted by the NaOH-urea solvent, arabinoxylan, (1:1) mixture of arabinoxylan and β -glucan, and β -glucan were dissolved in distilled water at 70 °C (40 mg/mL). Solutions were poured into flat dishes of 3 cm diameter and dried at 45 °C for 24 h. The films were cut into rectangular segments (5 × 20 mm) and their mechanical properties were investigated by the tensile tester (Tack Tester TA-500, UBM Co., Kyoto, Japan). Tensile test was conducted at a constant rate (0.01 mm/sec) at 25 °C. Strain data and tensile force were collected twice a second. Breaking stress (σ_{\max} , MPa) and maximum strain (ϵ_{\max} , %) were determined at the breaking point of the films. Elastic modulus E (GPa) was calculated from a linear region at the initial part of the strain-stress curve.

3. Results and Discussion

Chemical compositions (wt% in dry weight basis) including relative monosaccharide composition of CP are listed in Table 1. Hemicellulose and cellulose content in CP were 73.6% and 13.5%, respectively, indicating the abundance of hemicellulose in CP. The present distribution of hemicellulose and cellulose was similar to the values (67% and 18%) reported previously [13]. Beta-glucan content in CP (3.2%) was higher than that of corn grain (0.5-1.3%) [14], but was comparable to that of oat and barely (3-5%) [15]. Beta-glucan is a hemicellulose characteristic for monocots and is located mainly in the endospermic cell walls. Beta-glucan contained in cereal grains is worth mentioning because of its bioactivities [15], such as lowering blood cholesterol [16]. Monosaccharide composition of CP (Table 1) was similar to that of the maize bran which contained pericarp, tip cap and hull [1]. Total content of arabinose and xylose in CP amounted to 66.1% (wt%), showing abundance of arabinoxylan as the main hemicellulose in CP.

Table 1. Chemical compositions including relative monosaccharide composition of sweet corn pericarp

Component	wt% (dry weight basis)
Protein	5.0 ± 0.4
Lipid	4.9 ± 0.3
Ash	1.1 ± 0.1
Cellulose	13.5 ± 0.9
Hemicellulose	73.6 ± 0.4
Uronic acid	7.7 ± 0.1
?-Glucan	3.2 ± 0.1
Pectin	8.7 ± 1.5
Starch	3.5 ± 0.1
<i>Relative monosaccharide composition</i>	
Xylose	40.0 ± 0.3
Glucose	28.6 ± 0.8
Galactose	5.4 ± 0.7
Arabinose	26.1 ± 0.2

Values are expressed as mean ± SD ($n = 2$).

Solubility (wt%) of CP in 0-8% NaOH solutions containing 0-8 M urea and relative monosaccharide composition of the solubilized materials are listed in Figure 1. We first investigated the solubility of CP in 2 wt% NaOH containing 0-8 M urea (Figure 1a). The solubility increased with increase in urea concentration and reached a maximum (72.2%) at 6 M urea. Next, we tested the solubility of CP and

monosaccharide composition of the solubilized materials in 0-8% NaOH solutions containing 6 M urea (Figure 1a, c). Solubility increased with increase in NaOH concentration and reached a maximum at 2 wt% NaOH with 6 M urea (Figure 1b). Addition of urea clearly enhanced the solubility of CP below 4 wt% NaOH. No significant effect, however, was detected above 6 wt% NaOH.

The carbohydrate composition analysis indicates that the content of arabinoxylan in the solubilized fractions with NaOH containing 6 M urea increased with increase in NaOH concentration and attained 69.3% at 2 wt% NaOH (Figure 1c). The residue was, however, rich in cellulose (data not shown). Previously arabinose to xylose ratio (A/X) has been shown to affect the solubility of arabinoxylan [17]. With increasing the values of A/X, the solubility increased because of decreasing in inter- and intra-molecular hydrogen bonds [18]. Our results showed that the value of A/X changed from 0.84 to 0.72 by addition of 6 M urea in 2 wt% NaOH, suggesting that the NaOH-urea solvent system allowed extraction of widely branched arabinoxylans from CP. Solubility of β -glucan was also improved from 83.1% to 90.6% by the addition of 6 M urea in 2 wt% NaOH as evident from the higher glucose content of solubilized materials with urea (25.2%) than that without urea (14.9%), Figures 1c, d. Comparable data was previously reported by Bhatti [19] who showed that 81% of β -glucan was recovered from barley by extraction with 4% NaOH.

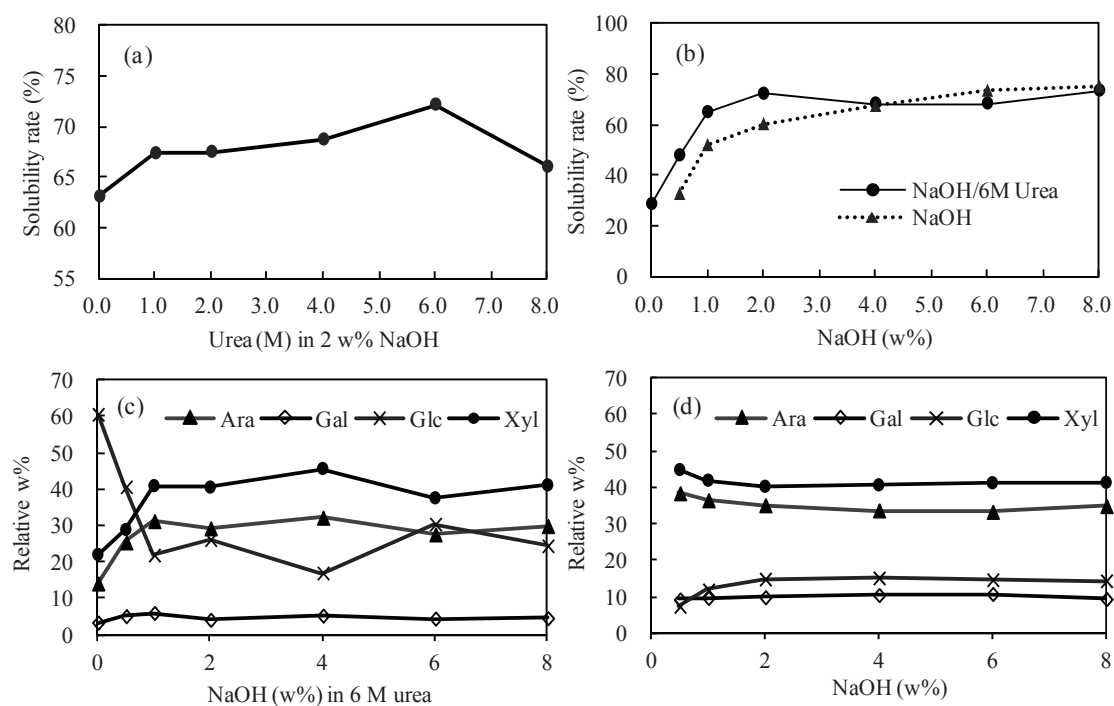


Fig. 1. Effects of urea (a) and NaOH (b) on the solubility of CP and the carbohydrate compositions of the materials solubilized by extraction with 0-8 w% NaOH-6 M urea (c) and 0.5-8 w% NaOH (d)

Typical LV-SEM images of the native CP and the residue after treatment with 2 wt% NaOH-6 M urea are shown in Figure 2. The native CP has thin fibriform structure. Although a large amount of CP was solubilized by the treatment, the residues after the treatment retained the original fibriforms accompanied by some cracks.

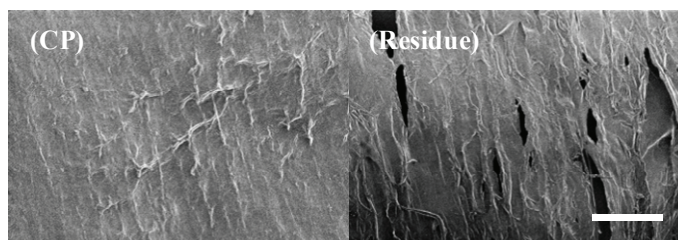


Fig. 2. Typical LV-SEM images of the native CP and the residue after treatment with 2 wt% NaOH-6 M urea. Scale bar represents 50 μm

The present study shows for the first time the effectiveness of the NaOH-urea solvent system for solubilization of hemicelluloses in CP. The NaOH-urea solvent system has been originally developed for solubilization of cellulose [7]. Cai *et al.* [20] proposed a solubilization mechanism that the formation of a urea and cellulose-NaOH complex at low temperatures allowed the rapid dissolution of cellulose. The optimal condition for solubilization of hemicelluloses in CP is 2 wt% of NaOH in 6 M urea as shown in the present study, differing from that of cellulose, which is 7 wt% NaOH-12 wt% urea-81 wt% water [7].

Table 2. Mechanical properties of biofilms

Source of films	σ_{max} (MPa)	ϵ_{max} (%)	E (GPa)
Arabinoxylan	46.3 ± 5.4	2.8 ± 0.8	3.30 ± 0.14
Extracted hemicellulose	56.2 ± 8.1	3.5 ± 1.7	3.09 ± 0.72
(1:1) Mixture of arabinoxylan and β -Glucan	60.5 ± 12.7	12.4 ± 3.7	2.30 ± 0.49
β -Glucan	72.5 ± 19.5	19.3 ± 4.5	1.93 ± 0.38

Values are expressed as mean \pm SD ($n = 5$).

Since the extracted hemicelluloses form transparent films after simple drying of their aqueous solutions, we subsequently analyzed mechanical properties of their films by the tensile test. The representative stress-strain curves are shown in Figure 3. The values of breaking stress (σ_{max}), maximum strain (ϵ_{max}) and elastic modulus (E) are summarized in Table 2. Both maximum breaking stress and maximum strain increased with increase in the β -glucan content, and showed 72.5 MPa and 19.3% in the 100% β -glucan film. Reverse relationship was, however, observed in the elastic modulus. Elastic modulus increased with increase in the arabinoxylan content, and showed 3.30 GPa in the 100% arabinoxylan film. These results suggest that β -glucan gives mechanical strength and plasticity to the arabinoxylan films. Arabinoxylan in the films contributes towards stiffness against deformation and brittleness. The differences observed in σ_{max} and ϵ_{max} might be attributed to the strength of intermolecular hydrogen bonds between polysaccharide chains. Beta-(1,3;1,4)-glucan, with about 70% of (1,4)-linked and 30% of (1,3)-linked D-Glcp residues, has been shown to have strong intermolecular hydrogen bonds along the (1,4)-linked units [15]. On the other hand, arabinoxylan has been shown to possess limited intermolecular hydrogen bonds because of steric hindrance of highly substituted arabinose residues [15]. Randomly dispersed β -(1,3)-Glcp units among the (1,4)-linked regions have been demonstrated to increase flexibility of the molecule [21]. The values of σ_{max} and ϵ_{max} for the extracted hemicellulose film containing 4.3% β -glucan were 1.2- and 1.3-fold higher than those of arabinoxylan alone. These results clearly show the improvement of physical strength and flexibility of arabinoxylan films by the addition of β -glucan. Izydorczyk and MacGregor [22] reported the existence of non-covalent interactions between arabinoxylan and β -glucan in barley. Recently

physicochemical properties of arabinoxylan films were also investigated for their practical utilization as an edible film [18,23,24]. Arabinoxylan film from corn hull has been shown to have 53.8 MPa of σ_{\max} , 6.2% of ϵ_{\max} and 1.32 GPa of modulus [23], while that in rye flour the values were 52.4 MPa of σ_{\max} , 4.7% of ϵ_{\max} and 1.75 GPa of modulus [18]. In addition, Stevanic *et al.* [24] reported an interesting result that addition of 5 % bacteria cellulose to arabinoxylan film improved the mechanical properties from 58 to 68 MPa of σ_{\max} and 2.5 to 2.7 GPa of modulus. These values of σ_{\max} data for the arabinoxylan films are slightly higher than those of the present arabinoxylan film, but are similar to the films prepared by the extracted hemicelluloses containing 4.3% β -glucan.

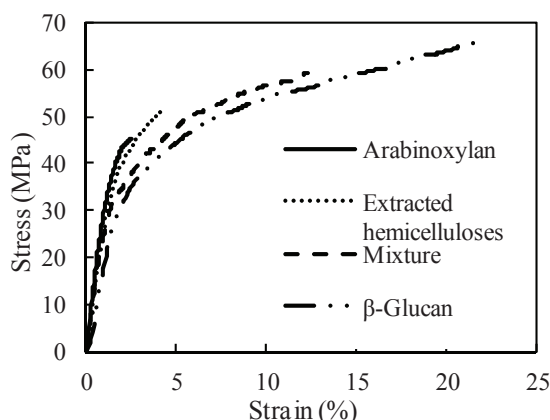


Fig. 3. Representative Stress-Strain curves of biofilms prepared from arabinoxylan, the extracted hemicellulose containing 4.3% β -glucan content, (1:1) mixture of arabinoxylan and β -glucan, and β -glucan.

4. Conclusion

The present study shows that the NaOH-urea solvent system is effective for the extraction of arabinoxylan mixed with β -glucan from CP and might be desirable for the utilization of the extracted materials as a source of biofilms.

References

- [1] Saulnier L., Marot C., Chanliaud E., Thibault J.F., *Carbohydr. Polym.* 1995; **26**: 279-287.
- [2] Yadav M.P., Parris N., Johnston D.B., Hicks K.B., *J. Agric. Food Chem.* 2008; **56**: 4181-4187.
- [3] Doner L.W., Hicks K.B., *Cereal Chem.* 1997; **74**: 176-181.
- [4] Vidal Jr B.C., Rausch K.D., Tumbleson M.E., Singh V., *Cereal Chem.* 2009; **86**: 133-135.
- [5] Allen S.G., Schulman D., Lichwa J., Antal Jr M.J., Laser M., Lynd L.R., *Ind. Eng. Chem. Res.* 2001; **40**: 2934-2941.
- [6] Dien B.S., Li X.L., Iten L.B., Jordan D.B., Nichols N.N., O'Bryan P.J., Cotta M.A., *Enzyme Microbiol. Technol.* 2006; **39**: 1137-1144.
- [7] Yoshida T., Tsubaki S., Teramoto Y., Azuma J., *Bioresour. Technol.* 2010; **101**: 7820-7826.
- [8] Cai J., Zhang L., *Biomacromolecules* 2006; **7**: 183-189.
- [9] Dervilly G., Saulnier L., Roger P., Thibault J.F., *J. Agric. Food Chem.* 2000; **48**: 270-278.
- [10] López-Casado G., Matas A.J., Dominguez E., Cuartero J., Heredia A., *J. Exp. Bot.* 2007; **58**: 3875-3883.
- [11] Filisetti-Cozzi T.M.C.C., Carpita N.C., *Anal. Biochem.* 1991; **197**: 157-162.
- [12] Seaman J.F., Bubl J.L., Harris E.E., *Ind. Eng. Anal. Ed.* 1945; **17**: 35-37.
- [13] McKee L.H., Latner T.A., *Foods Hum. Nutr.* 2000; **55**: 285-304.
- [14] Demirbas A., *Food Chem.* 2005; **90**: 773-777.

- [15] Ebringerova A., Hromadkova Z., Heinze T., *Adv. Polym. Sci.* 2005; **86**: 1-67.
- [16] Brennan C.S., Cleary L.J., *J. Cereal Sci.* 2005; **42**: 1-13.
- [17] Doner L.W., Chau H.K., Fishman M.L., Hicks K.B., *Cereal Chem.* 1998; **75**: 408-411.
- [18] Höije A., Sternemalm E., Heikkinen S., Tenkanen M., Gatenholm P., *Biomacromolecules* 2008; **9**: 2042-2047.
- [19] Bhatta R.S., *Cereal Chem.* 1993; **70**: 73-77.
- [20] Cai J., Zhang L., Liu S.L., Liu Y.T., Xu X.J., Chen X.M., Chu B., Guo X.L., Xu J., Cheng H., Han C.C., Kuga S., *Macromolecules* 2008; **41**: 9345-9351.
- [21] Wood P.J., Weisz J., Beer M.U., Newman C.W., Newman R.K., *Cereal Chem.* 2003; **80**: 329-332.
- [22] Izydorczyk M.S., MacGregor A.W., *Carbohydr. Polym.* 2000; **41**: 417-420.
- [23] Zhang P.Y., Whistler R.L., *J. Appl. Polym. Sci.* 2004; **93**: 2896-2902.
- [24] Stevanic J.S., Joly C., Mikkonen K.S., Pirkkalainen K., Serimaa R., Remond C., Toriz G., Gatenholm P., Tenkanen M., Salmen L., *J. Appl. Polym. Sci.* 2011; **122**: 1030-1039.